YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2014; January Vol. 71 (No. 1): p. 1-126.

YU ISSN 0042-8450 vol. 71, br. 1, 2014.

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

Adresa redakcije: Vojnomedicinska akademija, Institut za naučne informacije, Crnotravska 17, poštanski fah 33–55, 11040 Beograd, Srbija. Telefoni: glavni i odgovorni urednik 3609 311, glavni menadžer časopisa 3609 479, pretplata 3608 997. Faks 2669 689. E-mail (redakcija): **vsp@vma.mod.gov.rs**

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

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 1227423129552141 5. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € (u dinarskoj protivvrednosti na dan uplate) za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa Vojna štamparija, Beograd, Resavska 40 b.

YU ISSN 0042-8450 vol. 71 No. 1, 2014

VOJNOSANITETSKI PREGLED

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

PUBLISHER
Military Health Department, Ministry of Defence, Serbia

Editorial Office: Military Medical Academy, INI; Crnotravska 17, PO Box 33–55, 11040 Belgrade, Serbia. Phone: Editor-in-chief +381 11 3609 311; Main Journal Manager +381 11 3609 479; Fax: +381 11 2669 689; E-mail: **vsp@vma.mod.gov.rs**

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

The Journal is published monthly. Subscription: Giro Account No. 840-314849-70 Ministry of Defence – Total means of payment – VMA (for the Vojnosanitetski pregled), refer to number 12274231295521415. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40 b.

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

2014 – The year of jubilees of the Serbian Military Medical Corps

Godina 2014 – godina jubileja u sanitetskoj službi Vojske Srbije

Silva Dobrić

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We shall certainly remember the new year 2014 especially significant for the Military Medical Corps of the Republic of Serbia. In fact, the year 2014 marks 175 years of the establishment of organized medical service in the Serbian Army, 170 years of the establishment of the Central Military Hospital, whose tradition has been continued by the Military Medical Academy (MMA) in Belgrade, and 70 years of publishing the first issue of the Vojnosanitetski Pregled (VSP), an official scientific journal of physicians, dentists and pharmacists of the Serbian Army. On these anniversaries, throughout the year, especially in the months and on the days that are related to these events (March 2 – the Day of the Military Medical Academy, July 30 – the Day of Armed Forces Medical Services of Serbia, and September, the month in which in 1944 the first issue of VSP was printed) a numerous scientific, professional and other suitable events to will be held to look at all the aspects of Serbian Military Medical Service in details, its professional, scientific and educational activities for which Serbia is known worldwide. More words will be written on the pages of the VSP within the whole year. Among other things, all aspects of the development of the Journal over the past 70 years will be particularly analyzed and discussed on the possibilities of further improvements. On this occasion, as before, a short overview of the Journal's Editorial Office work in the past year will be given.

Over the 2013 (to December 15 inclusive) the Editorial Office of the VSP received 258 manuscripts, most befalling to the category Original Articles (134 or 51.9%) and Case Reports 74 or 28.7%). Of these papers, 118 (45.7%) have been already evaluated by reviewers (out of that, 64% accepted for publication after correction, and 36% rejected), 33 (12.8%) marked as inappropriate without sending to reviewers, while the remaining 107 (41.5%) are under the reviewing process. A reduced number of papers accepted for publishing in 2013, compared to 2012, when we received 281 manuscripts, or in 2011 324 ones, is certainly a consequence of the Journal's Editorial Board policy to further review only manuscripts closely related to the main topics covered by the Journal, above all clinical medicine, as well as the fact that the manuscripts recieved have to be

Nova 2014. godina biće, svakako, upamćena kao godina od posebnog značaja za vojni sanitet Republike Srbije. Naime, ove godine navršava se 175 godina od uspostavljanja organizovane sanitetske službe u Vojsci Srbije, 170 godina od osnivanja Centralne vojne bolnice, čiju tradiciju nastavlja Vojnomedicinska akademija (VMA) u Beogradu, i 70 godina od početka izlaženja časopisa „Vojnosanitetski pregled" (VSP), naučnog i stručnog glasila lekara, stomatologa i farmaceuta Vojske Srbije. Tim povodom, tokom cele godine, a posebno u mesecima i danima koji se vezuju za navedene događaje (2. mart – Dan VMA, 30. jul – Dan sanitetske službe Vojske Srbije i septembar, mesec u kome je 1944. godine odštampan prvi broj VSP-a) predviđeno je održavanje niza naučnih, stručnih i drugih prigodnih manifestacija, u okviru kojih će se podrobnije sagledati svi aspekti vojnomedicinske stručne, naučne i obrazovne delatnosti u našoj zemlji, delatnosti po kojima je Srbija poznata širom sveta. O ovim događajima biće tokom godine više reči i na stranicama našeg časopisa. Između ostalog, posebno će biti analizirani svi aspekti razvoja časopisa tokom proteklih 70 godina i razmotrene mogućnosti daljeg poboljšanja. Ovom prilikom, kao i do sada, biće dat kratak osvrt na rad Redakcije časopisa u protekloj godini.

Tokom 2013. godine (zaključno sa 15. decembrom) u Redakciju VSP-a pristiglo je 258 radova, najviše iz kategorija Originalni radovi (134 ili 51,9%) i Prikazi bolesnika (74 ili 28,7%). Od ovih radova 118 (45,7%) je već recenzentski obrađeno (od njih je 64% nakon učinjenih korekcija prihvaćeno za publikovanje, a 36% je odbijeno), 33 (12,8%) su arhivirana kao neodgovarajuća bez prethodnog slanja recenzentima, dok je preostalih 107 (41,5%) još u postupku recenzije. Smanjenje broja primljenih radova u odnosu na 2012, kada je primljen 281 rad ili 2011, kada su primljena 324 rada, sigurno je posledica odluke Uredništva da se u dalji proces recenzije uzimaju samo radovi koji se uže odnose na oblast koju časopis pokriva, a to je, pre svega, klinička medicina, kao i činjenice da se primaju isključivo rukopisi na engleskom jeziku (izuzetak su pojedini prikazi iz kategorije Istorija medicine i U fokusu, kada je zbog navođenja citata iz izvornih do-

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written in English (with the exception of some papers from the category of the History of Medicine and In Focus, e.g. the quotations from the original documents in our language, and themes specific for the local professional community, respectively, that are more appropriate to be published in Serbian). However, this number of received manuscripts is still very high for our conditions, and allows regular publishing of the Journal with 13 to 14 articles from different categories monthly.

Analysis of submitted papers by the institutions of their authors, showed that 91.5% of the papers came from the so-called 'civilian academic and scientific institutions', of which about 8% was from abroad, confirming once again that the VSP in recent years, especially since it has been selected for indexing in Thomson Reuters Science Citation Index Expanded (SCIE) database and got impact factor, has ranked itself a Journal of the most widely medical professional and scientific community not only in Serbia but abroad, as well.

In the past year, 191 articles, including 3 book reviews and 2 scientifing meeting reports, were published by the VSP (Table 1). As before, the majority of the articles were classified as Original Articles (52%) and the category Case Reports (25.1%), which is proportional to the number of manuscripts received from these categories, not only, as already said, in 2013 but in the previous years, as well. It should also be noted that within 2013, each month, in addition to the printed version of each issue of the Journal, 4-5 articles were published On-Line-First with a DOI number [these articles are available to readers at the website of the Journal and *via* DOISerbia service, accessed through the site of the Consortium of Libraries of Serbia for Coordinated Acquisition (KoBSON)]. Next, after some time, these articles are "staked down" and published printed by the VSP, but with the same DOI number.

kumenta na našem jeziku, odnosno tema koje su ciljano usmerene ka domaćoj stručnoj javnosti, prikladnije te priloge objaviti na srpskom jeziku). Međutim, i ovaj broj pristiglih rukopisa je, za naše prilike, izuzetno visok, i omogućava nesmetano jednomesečno izlaženje časopisa sa 13 do 14 članaka iz različitih kategorija.

Analiza pristiglih radova prema institucijama njihovih autora, pokazuje da je 91,5% radova stiglo iz tzv. civilnih akademskih i naučnih institucija, od čega oko 8% iz inostranstva, što još jednom potvrđuje da je VSP poslednjih godina, pogotovo otkako je ušao u sistem praćenja baze Science Citation Index Expanded (SCIe) i dobio impakt faktor, postao časopis najšire medicinske stručne i naučne javnosti ne samo Srbije, već i inostranstva.

U protekloj godini, na stranicama VSP-a objavljen je ukupno 191 članak, uključujući i tri prikaza kjiga i dva izveštaja sa stručnog skupa (Tabela 1). Kao i prethodnih godina, najveći broj objavljenih članaka pripadao je kategoriji Originalni članci (52%) i onima iz kategorije Prikaz bolesnika (25,1%), što je u skladu sa brojem primljenih radova iz tih kategorija, ne samo, kako je napred navedeno u 2013. godini, nego i u ranijim godinama. Treba istaći, da je i tokom 2013. godine, svaki mesec uz štampani broj časopisa bilo objavljeno 4-5 radova elektronski kao OnLine-First, sa pripadajućim DOI brojem i ti radovi bili su dostupni čitaocima preko sajta časopisa i servisa DOISerbia, kome se može pristupiti preko sajta Konzorcijuma biblioteka Srbije za objedinjenu nabavku (KoBSON). Ti radovi su, potom, nakon izvesnog vremena „skidani" i objavljivani u štampanoj verziji časopisa, ali sa istim DOI brojem.

Table 1

Categories and the number of articles published in the Vojnosanitetski Pregled in 2013/ Kategorije i broj članaka objavljenih u Vojnosanitetskom pregledu u 2013.

Category / Kategorija	Articles/ Članci	
	n	%
Editorial/ Uvodnik	5	2.7
Original Article/ Originalni članak	99	52
General Review/ Opšti pregled	8	4.2
Current Topic/ Aktuelna tema	10	5.3
Practical Advice for Physicians/ Seminar praktičnog lekara	4	2.0
Case Report/ Prikaz slučaja	48	25.1
Preliminary Report/ Prethodno saopštenje	1	0.5
Short Communication/ Kratko saopštenje	1	0.5
History of Medicine/ Istorija medicine	5	2.7
In Focus/ U fokusu	2	1.0
Letter to the Editor/ Pismo uredniku	3	1.5
Book Review/ Prikaz knjige	3	1.5
Scientific Meeting Report/ Izveštaj sa stručnog skupa	2	1.0
Total/ Ukupno	191	100.0

When analyzing the articles (including book reviews and scientific meeting reports) published by the VSP in 2013 considering the authors' affiliations, one can notice that the largest number of the authors, just as it was in the years before, were from 'civilian institutions', domestic and foreign

S obzirom na institucije autora čiji su radovi objavljeni u VSP-u u toku protekle godine, ponovo je, kao i prethodnih godina, najveći broj objavljenih radova (uključujući i prikaze knjiga i izveštaje sa stručnih skupova) bio od autora iz civilnih institucija, domaćih i stranih (61%), zatim slede zajednič-

(61%), followed by the co-authors from both civilian and military medical institutions (27.5%), while the least number of the authors were from the MMA and other military medical institutions (11.5%). Regarding the articles written by the foreign authors or co-authors, they account for 12.5% in 2013.

Thanks to receiving manuscripts through the electronic system, here introduced in 2012, also allowing checking submitted papers to plagiarism/selfplagiarism we successfully prevented many times publication of articles with a high percentage of overlapping text with already published articles. This software, however, recognizes English only, so that the members of the VSP Editorial Staff pay additional efforts to detect eventual plagiarism/selfplagiarism articles published in Serbian earlier. Devoted to the highest ethical principles in the publishing we, certainly, go on honestly expecting also to justify the high reputation of the Journal and increase its impact on the international level. The fact that the impact factor of the Journal increased by about 17% (from 0.179 to 0.21) last year, confirms that we go the right way. So, let us believe this tendency will go on further.

To come to this goal, we also lean on our reviewers who really do their best regarding such a high number of submitted manuscripts. All of them are listed in Table 2.

On behalf of the Publisher (Military Health Department of the Ministry of Defence), the Editorial Board and the membres of the Editorial Staff of the Journal, as always so far, I thank them all sincerely, from the bottom of my heart, for their efforts wishing that our successful cooperation will go on to our mutual satisfaction.

ki radovi autora iz civilnih i vojnozdravstvenih institucija, uglavnom iz VMA (27,5%), dok je najmanje radova bilo od autora iz VMA i drugih vojnozdravstvenih centara (11,5%). Ako se posebno analiziraju radovi čiji su autori, odnosno koautori iz inostranstva, njih je među objavljenim radovima u 2013. godini bilo 12,5%.

Zahvaljujući primanju rukopisa preko elektronskog sistema, koji smo uveli 2012. godine, pomoću koga je moguća provera pristiglih radova na plagijarizam/autoplagijarizam, uspeli smo u više navrata sprečiti eventualno objavljivanje radova kod kojih je ustanovljen visok procenat preklapanja teksta sa već objavljenim radovima. Nažalost, softver koji vrši tu proveru, programiran je samo za prepoznavanje teksta na engleskom jeziku, tako da članovi Redakcije časopisa ulažu dodatne napore da ustanove eventualni plagijarizam/autoplagijarizam primljenih radova u odnosu na prethodno objavljene radove na srpskom jeziku. Nastavak borbe za uspostavljanje najviših etičkih principa u publikovanju biće i dalje jedan od naših prioriteta, čime ćemo opravdati visok renome časopisa i povećati njegovu uticajnost u međunarodnim okvirima. Da smo na dobrom putu, svedoči i podatak da nam je u prošloj godini impakt faktor porastao za oko 17% (sa 0,179 na 0,21), a nadamo se da će se taj trend nastaviti i ubuduće.

U tome veliku pomoć očekujemo od naših recenzenata koji, s obzirom na ovako visok priliv radova, iz godine u godinu urade ogroman posao. Imena onih koji su bili angažovani u recenziranju radova u protekloj godini data su u Tabeli 2. Njima se, u ime Izdavača (Uprava za vojno zdravstvo), Uredništva časopisa i cele Redakcije, kao i uvek do sada, od srca zahvaljujem na uloženom trudu s nadom da ćemo i u 2014. godini nastaviti sa uspešnom saradnjom na obostrano zadovoljstvo.

Table 2

Reviewers of the Vojnosanitetski pregled in 2013 / Recenzenti Vojnosanitetskog pregleda u 2013. godini

Ač Nikolić Eržebet
Aleksić Petar
Aleksić Predrag
Andrén-Sandberg Åke
Antić Branislav
Antonijević Biljana
Arsović Nenad

Balestra Constantino
Baletić Nenad
Balint Bela
Bančević Vladimir
Bejeh-Mir Arash Poorsattar
Berisavac Milica
Beslać Bumbaširević Ljiljana
Bjegović Mikanović Vesna
Bjelović Miloš
Bojanić Nebojša
Bokonjić Dubravko
Božić Marija
Brennan Katie Kelly
Brkić Zlata
Bulat Petar
Bumbaširević Marko
Cerović Snežana
Comella Cynthia L.
Czaja Kryzstof

Čekanac Radovan
Čolić Miodrag

Ćuk Vladimir
Ćurčić Marijana

Daković Dragana
Damjanov Nemanja
Davidović Lazar
De Broe Marc E.
De Chadarévian Jean-Pierre
Dedić Gordana
Dimitrijević Jovan
Dimković Nada
Dinčić Dragan
Dobrić Silva
Doder Radoje
Dost Axel
Dragojević Simić Viktorija
Dragović Tamara
Đorđević Snežana
Đuričić Slaviša
Đurović Aleksandar
Đurović Branislav
Đurović Branka

EL-Hossiny Hesham

Gajić Stevanović Milena
Gazivoda Dragan
Gershlick AH
Glišin Vladimir
Goldsmith Stanly
Gržetić Ivan
Guć-Šćekić Marija

Hajduković Zoran
Hassan Neven

Ignjatović Mile
Ignjatović Svetlana
Ilić Tihomir
Jakovljević Mihajlo
Janković Borisav
Janković Slobodan
Jovanović Dragana
Jovanović Marina
Jovanović Milan
Jović Jasna
Jović Nebojša
Jović Stošić Jasmina
Jovičić Bojan

Kandolf Sekulović Lidija
Khalid Sakib
Konstantinović Ljubica
Koračević Goran
Kostić Vladimir
Kostov Miloš
Kot Jacek
Kozarski Jefta
Kozomara Ružica
Krivokapić Zoran
Kumar Anil

LaFranchi Stephen
Lakić Aneta

Laowattana Somchai
Lazić Miodrag
Lazić Zoran
Lečić Toševski Dušica
Lepić Toplica
Lepšanović Zorica
Lončar Dragan

Ljubić Aleksandar

Magić Zvonko
Majkić Singh Nada
Maksić Đoko
Mandić Gajić Gordana
Marić Nađa
Marjanović Ivan
Marjanović Marjan
Marković Dejan
Marković Svetomir
Martić Vesna
Matić Smiljana
Matijević Stevo
Medenica Ivica
Mi Jie
Micić Dragan
Mičić Sava
Mihaljević Biljana
Mijušković Željko
Mikić Dragan
Milanović Slađan
Mileusnić Dušan
Mirković Darko
Mirković Ljiljana
Mirović Veljko
Mitrović Jovanović Ana
Morawska Lidia
Mulcahy Levy Jean
Nakamura A.
Nedok Aleksandar
Nežić Duško
Nikolić Branka
Nikolić Dragan
Nikolić Ljubiša
Ninković Milica
Noor Shehla

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Obradović Slobodan
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O'Neill Iain
Opinćal Stošić Tatjana
Ostojić Gordana

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Pašić Srđan
Pavlović Milorad
Perez-Bóscollo Adriana Cartafina
Perišić Nenad
Peško Predrag
Petakov Milan
Petković Stevan
Polovina Marija
Popović Zoran
Poštić Srđan
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Radlović Nedeljko
Radojčić Ljiljana
Radonjić Vidosava
Radosavljević Davorin
Radosavljević Vladan
Rađen Slavica
Raičević Ranko
Ristić Anđelka
Ritter Robert C.
Roganović Zoran
Romić Predrag
Rudnjanin Slobodan

Sakhuja R.
Sanders Jean
Sazdanović Predrag
Sibel Mentese
Simić Radoje
Simić Snežana
Slavković Slobodan
Stamatović Dragana
Stamatović Novak
Stamenković Dušica
Stančić Ivica
Stanković Goran
Stanković Nebojša
Stefanović Dara
Stefanović Dušan
Stepanović-Petrović Radica
Stojanović Dušica
Stojanović Paović Anka

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Šipetić Grujičić Sandra
Šobajić Slađana
Šuljagić Vesna
Šurbatović Maja
Šušnjar Snežana

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Tarabar Dino
Tarabar Olivera
Tatomirović Željka
Terzić Milan
Thalange N.
Todorić Milomir
Todorović Ljubomir
Todorović Zoran
Tomasi Paolo A.
Tomić Aleksandar
Topić Aleksandra
Toševski Jovo
Tošović Slobodan
Tukić Ljiljana
Tulić Cane

Ušaj Knežević Slavica

Vanberg Paul
Veljančić Dragana
Verma Rohit
Videnović Aleksandar
Vojvodić Danilo
Vrbić Miodrag
Vučević Dragana
Vučinić Slavica
Vučinić Žarko
Vukomanović Aleksandra

Zečević Radoš
Zoranović Uroš

Životić-Vanović Mirjana
Žunić Gordana

ORIGINAL ARTICLES

UDC: 616.314-089.23
DOI: 10.2298/VSP120629033N

Changes in soft tissue profile following the treatment using a Herbst appliance – A photographic analysis

Promena mekotkivnog profila posle terapije Herbst aparatom – analiza fotografija

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Abstract

Background/Aim. Aesthetics is the reason for most of the class II malocclusion patients to opt for orthodontic treatment. In order to regulate retrognathic profile Herbst appliance for anterior movement of the mandible can be a treatment of choice. The aim of this study was to determine the soft tissue profile changes following Herbst appliance therapy on before and after treatment photos, using the computer program. **Methods.** This investigation was performed on profile photographs of 20 class II patients (12 females and 8 males) aged 18–23 years. Analysis of the changes in soft tissue facial structure relationships evident on the photographs before and after the Herbst appliance therapy was performed using Bentley Micro Station program. The first contour of the soft tissue profile was marked. The following reference lines were subsequently traced: Ricketts aesthetic E line and Juanita line. The area enclosed by these two lines included the nose, upper and lower lip, chin and free space in front of the lips. Using the computer program, the surfaces of the soft tissue structures and free space defined by the reference lines and profile contours were measured. Calculation of the relative proportion of surfaces was done for each photograph. The data obtained were then compared for each patient before and after the treatment. Skeletal and dentoalveolar treatment effects that support soft tissue changes were presented by the profile cephalometric parameters of sagital occlusion (SO) analysis. **Results.** A reduction in the relative surface of the upper lip in males ($p < 0.01$) and females ($p < 0.05$) was shown by the pictures. The space occupied by the chin was reduced after the treatment for females only ($p < 0.05$). The relative surface of the nasal soft tissues, that was included in the reference space was increased ($p < 0.01$) in both genders. The relationship between the soft tissue and empty surface was changed in favor of the empty surface ($p < 0.05$) in females. No statistically significant differences were found between the males and the females, before or after the Herbst appliance therapy. The soft tissue changes were the consequence of skeletal and dentoalveolar treatment effects: upper incisors retrusion, lower incisor protrusion and forward movement of the lower jaw. **Conclusions.** The Herbst appliance therapy caused a significant improvement of the profile appearance. The extent of soft tissue changes that occur on its dentoalveolar and skeletal support is a complex issue.

Key words:
malocclusion, angle class II; orthodontics, corrective; photography; anthropometry; face.

Apstrakt

Uvod/Cilj. Većina mladih sa malokluzijom klase II odlučuje se za ortodontski tretman iz estetskih razloga. Za ispravljanje retrognatog profila može se koristiti aparat za anteriorno pomeranje mandibule – Herbst aparat. Cilj istraživanja bio je da se utvrde promene mekotkivnog profila posle primene Herbst aparata. **Metode.** Ispitivanje je izvršeno na profilnim fotografijama 20 mladih ljudi (12 ženskog i 8 muškog pola) sa malokluzijom klase II, starosti od 18 do 23 godine**.** Analizirane su promene odnosa mekotkivnih struktura lica na fotografijama načinjenim pre i posle sprovedene terapije Herbst aparatom. Za tu analizu korišćen je Bently Micro Station program. Digitalizovana je granica kontura mekotkivnog profila i ucrtavane su referentne linije: Ricketts-ova estetska E linija i Juanita linija. Ove linije ograničavaju prostor u kome su smešteni nos, gornja i donja usna i brada, kao i slobodni prostor koji se nalazi ispred konture mekotkivnog profila. Kompjuterski su merene površine mekotkivnih struktura i slobodnog prostora definisane referentnim linijama i konturom profila. Unutar slike izračunati su relativni odnosi površina. Nakon toga dobijeni podaci su poređeni pre i posle terapije za svaku osobu. Skeletni i dentoalveolarni terapijski efekti koji prouzrokuju mekotkivne promene prikazani su analizom sagitalne okluzije (SO) profilnog telerendgena. **Rezultati.** Sprovedenim merenjima utvrđeno je da je došlo do smanjenja relativne površine gornje usne kako kod dečaka ($p < 0,01$), tako i kod devojčica

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($p < 0,05$). Prostor koji zauzima brada posle terapije značajno se smanjio samo kod devojčica ($p < 0.05$). Relativna površina mekotkivnog profila nosa obuhvaćena referentnim prostorom povećana je sa podjednakom statističkom značajnošću od $p < 0,01$ kod oba pola. Odnos mekog tkiva i praznog prostora promenio se u korist praznog prostora ($p < 0,05$) kod devojčica. Nije bilo statistički značajne razlike između dečaka i devojčica pre i posle sprovedene terapije. Retruzija gornjih i protruzija donjih sekutića, kao i mezijalno pomeranje donje vilice glavni su terapijski efekti koji izazivaju mekotkivne promene donje trećine lica. **Zaključak.** Terapija Herbst aparatom dovodi do značajnog poboljšanja izgleda profila lica**.** Prilagodljivost mekog tkiva na promene njegove koštane i dentoalveolarne potpore je kompleksna.

Ključne reči:
malokluzija, klase II; ortodoncija, korektivna; fotografija; antropometrija; lice.

Introduction

Aesthetics is one of the main reasons for most of the patients with class II malocclusion, especially teenagers, to opt for orthodontic therapy expecting it to improve their features and thereby improve their self-confidence and acceptance amongst their peers [1–3]. In order to regulate retrognathic profile an appliance for anterior movement of the mandible, known as Herbst appliance (Bite Jumping Hinge appliance), can be the treatment of choice. Skeletal retrusion is characterized by convex profile that disturbs the facial appearance of a patient. It is considered that the morphology of the maxilla, mandible as well as the shape of the teeth has an indirect effect on the appearance of the face [4]. The appliance constantly holds the mandible in a protruded position and due to this activates the masticatory muscles.

It has been proved that compared to removable appliances fixed functional appliances can stimulate growth of the mandible and have a tendency to instigate horizontal growth of the condyle [5]. It also causes dentoalveolar changes, such as protrusion of mandibular incisors and retrusion of maxillary incisors which can have an effect on lip profile [6].

Many studies have shown that for the majority of cases the changes in soft tissue structures are not followed by the changes in hard tissues [7, 8]. However, changes in soft tissue structures represent a crucial parameter for evaluating the aesthetics following treatment. Most scientific papers on the effect of therapy with functional appliances emphasize the skeletal and dental changes, while only a few study the effects of treatment on the soft tissues [6]. There is a lack of long-term studies about the effects of Herbst appliance treatment while a clinical significance of soft tissue changes has been questioned [6]. In order to quantify soft tissue profiles and emphasize the importance of soft tissue profile assessment Skinazi et al. [9] measured the actual and relative size of the facial profile component parts instead of the common usage of the relationship between surface landmarks.

The aim of this study was to determine the soft tissue profile changes of class II cases following Herbst appliance therapy on pre- and aftertreatment photos, using the computer program and the present main skeletal and dentoalveolar treatment effects.

Methods

After Ethics committee approval and signing information consent the study was performed using profile photos taken before and after the treatment of patients with class II malocclusions using Herbst appliances at the Clinic of Orthodontics, Faculty of Dentistry, University of Belgrade.

All photographs were taken under standardized conditions (distance and position) with the same digital photo camera (Canon Power Shot G6). The distance was of 1.2 m and patients were sitting in the upright position with their heads stabilized in order to achieve the same position when taking photos before and after the treatment.

A total of 20 patients of both genders, average 20 years of age participated in this study (12 females from 18 to 22.5 years, and 8 males from 18.5 to 23 years). Skeletal maturity was first determined for each patient according to the stages of the cervical vertebral maturation. All of the patients indicated the stage of maximal growth, so the sample consisted of nongrowing patients.

Analysis of the changes in soft tissue facial structure relationships evident on the photographs before and after the Herbst appliance therapy was performed using Bentley Micro Station program. This program was used for the measurement of confined surfaces. I/RAS C (Intergraph) program was used to enter the digital photographs into the Bentley Micro Station program.

Bentley Micro Station program helped in marking the contour of the soft tissue profile. The following reference lines according to Skinazi et al. [9] were subsequently traced: Ricketts aesthetic E-passing through the soft tissue pogonion (Pg') and the pronazale (Pn) points; Juanita line passing through the subnasal (Sn) and supramentale (Sm) points [9] (Figure 1).

These points were used only for the purpose of defining the space within which the soft tissue structures were analyzed using measurements of relative surfaces.

The area enclosed by these two lines included the nose, upper and lower lip, chin and free space in front of the lips. Using the functions of the Micro Station program, the surfaces of the soft tissue structures and free space, defined by the reference lines and profile contours were measured. Calculation of the relative proportion of surfaces was performed for each photo. The data obtained were compared for each patient before and after the treatment.

All of the class II patients were treated successfully (class I molar relationship, normal overjet and overbite) at the end of the treatment. The therapy with the Herbst appliance and its effects were considered as combined, because the Herbst appliance was used in combination with the multibracket appliance (Figure 2). The Herbst appliance was

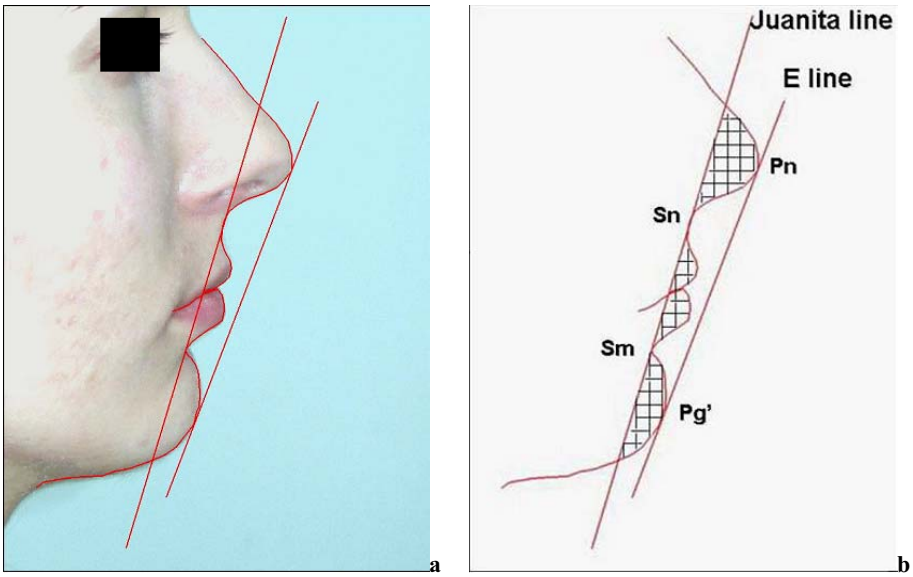


Fig. 1a – Juanita line, Ricketts aesthetic E line; b) the area enclosed by these two lines (nose, upper and lower lip, chin and free space in front of the lips).

removed after 6–8 months and then the multibracket fixed appliance was used, so the mean overall treatment time was 17 months. All of the patients were in the retention period. Recall visit for reevaluation of the treatment outcome was appointed in two years.

Fig. 2 – The cemented Herbst appliance and the attached fixed multibracket appliance on the frontal teeth.

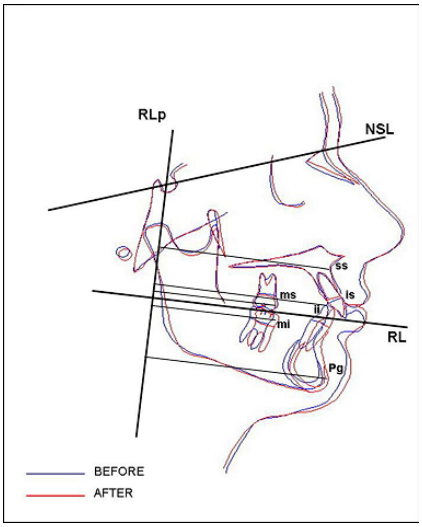


Fig. 3 – Superimposition of cephalometric images before (blue) and after (red) the treatment with visible skeletal, dentoalveolar and soft tissue changes.

Soft tissue changes caused by the Herbst appliance are the consequence of skeletal and dentoalveolar changes. In order to facilitate understanding this relation, it is necessary to present profile cephalometric parameters. SO analysis by Pancherz and Anebus-Pancherz [4] showed main sagittal skeletal and dentoalveolar changes. The pretreatment and posttreatment cephalometric images were first superimposed, in relation to the nasion-sella line (NSL), and, then, the maxillar occlusal plane – RL (occlusal reference line passing through the incisal edge of the upper incisor and the most distal point of molar contact in the occlusion) was determined. A line perpendicular to the RL through the sella (point S), *ie* RLp was used in measurements (Figure 3). Linear measurements were performed parallely with RL to RLp for each patient and selected in SO analysis (analysis of changes in sagittal occlusion), through the following parameters: ms-RLp – position of the first permanent maxillary molar (the shortest distance of the most mesial point of the approximal surface of the first upper molar to RLp); mi-RLp – position of the first permanent mandibular molar (the shortest distance of the most mesial point of the approximal surface of the lower first molar to RLp); (ms-RLp) – (mi- RLp) the molar relationship correction; is-RLp – position of the central maxillar incisor (the shortest distance of the incisal edge of the upper incisor to RLp); ii-RLp – position of the central mandibular incisor (the shortest distance of the incisal edge of the lower incisor to RLp); (is-RLp) – (ii-RLp) the overjet correction; ss-RLp – position of the maxilar base (the shortest distance of the most recessed point of the anterior side of the maxilla to RLp); Pg-RLp – position of the mandibular base

(the shortest distance of the most prominent point of the chin profile to RLp); (ss-RLp) – (Pg-RLp) the skeletal correction.

The results were statistically analyzed, using Microsoft Office Excel 2007. The used standard statistical analyses were: measures of central tendency – mean, measures of variability – standard deviation (SD), and statistical significance (p) of the obtained differences were shown by the Student's paired t-test.

Results

Table 1 shows the values of statistical reduction in the relative surface of the upper lip in the males ($p < 0.01$) and the females ($p < 0.05$). The relative surface of the lower lip was increased, but with no statistical significance in both genders (Figure 4). The space occupied by the chin was statistically significantly reduced after the treatment for the females only ($p < 0.05$). The nasal soft tissues relative surface, included in the reference space was statistically significantly increased ($p < 0.01$) in both genders. The relationship between the soft tissue and empty surface was changed in favor of the empty surface, and it was statistically significant ($p < 0.05$) in the females (Table 2). T-tests showed no statistically significant differences between the males and the females, before or after the Herbst appliance therapy.

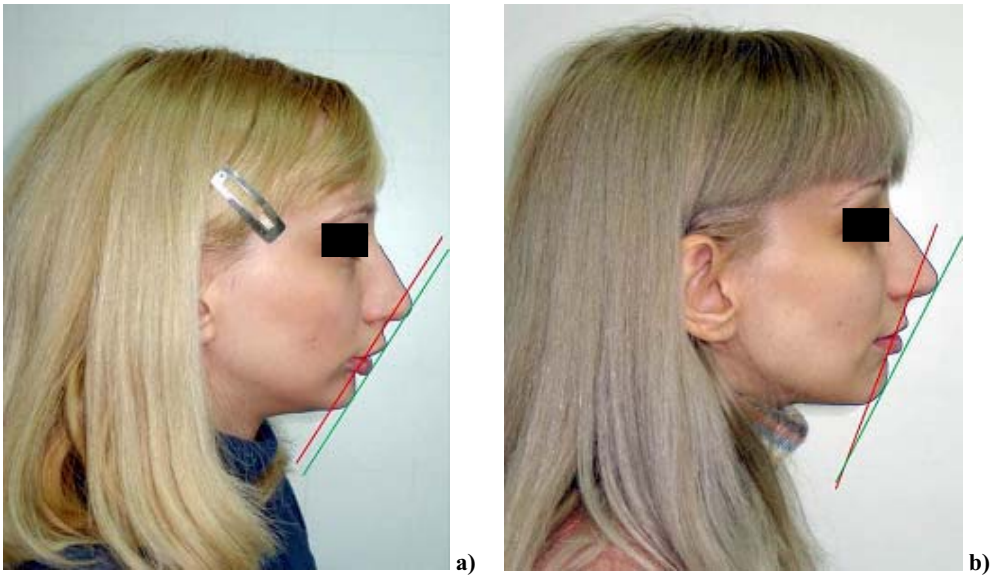
Main skeletal and dentoalveolar treatment changes are shown in Table 3, and Figure 5 and 6. The first permanent maxillar molar was distalized, whereas the first mandibular molar showed mesial position. Molar relation correction during the Herbst treatment was over 4 mm. The maxillar incisors showed retroinclination, whereas the mandibular ones showed proclination. Overjet correction during the treatment

Table 1

Distribution of the values before and after the Herbst treatment of class II cases for the percentage contribution of profile structures and the free space between the Ricketts and Juanita lines

Profile sturctures	Males (n = 8)			Females (n = 12)			t-test (males/females)	
	before (%) ($\bar{x} \pm SD$)	after (%) ($\bar{x} \pm SD$)	t-test	before (%) ($\bar{x} \pm SD$)	after (%) ($\bar{x} \pm SD$)	t -test	before	after
Nose	35.54 ± 9.13	50.45 ± 10.03	p < 0.01*	37.12 ± 8.64	50.76 ± 11.14	p < 0.01*	p = 0.704	p = 0.952
Upper lip	18.4 ± 4.28	11.36 ± 4.06	p < 0.01*	15.6 ± 3.77	11.19 ± 3.82	p < 0.05*	p = 0.149	p = 0.952
Lower lip	12.59 ± 4.41	13.54 ± 5.08	p = 0.697	12.32 ± 5.64	12.79 ± 3.28	p = 0.814	p = 0.912	p = 0.701
Chin	33.47 ± 11.50	24.65 ± 9.02	p = 0.110	34.95 ± 8.03	25.26 ± 8.12	p < 0.05*	p = 0.745	p = 0.878

***statistically significant**



a) b)

Fig. 4 – Before (a) and after (b) the treatment with Herbst apliance (the area between the Juanita - red and the Ricketts aesthetic E lines – green).

Table 2

The relative ratio of the surfaces filled with soft tissue structures of the profile and the empty space before and after the Herbst treatment of class II cases between the Ricketts and Juanita lines

Time related to the Herbst treatment	Soft tissue: free space ratio ($\bar{x} \pm SD$)		t-test
	male	female	
Before	1.73 ± 0.53	1.42 ± 0.28	p = 0.124
After	1.35 ± 0.31	1.21 ± 0.13	p = 0.190
t-test	p = 0.109	p < 0.05*	

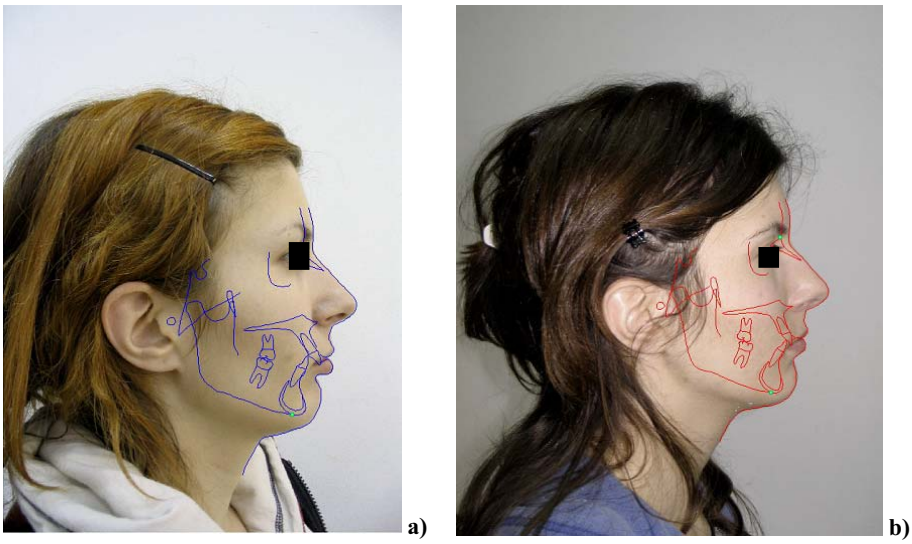
***statistically significant**

Table 3

Analysis of sagittal skeletal and occlusal changes before and after the treatment ($\bar{x} \pm SD$) – sagital occlusion (SO) analysis by Pancherz

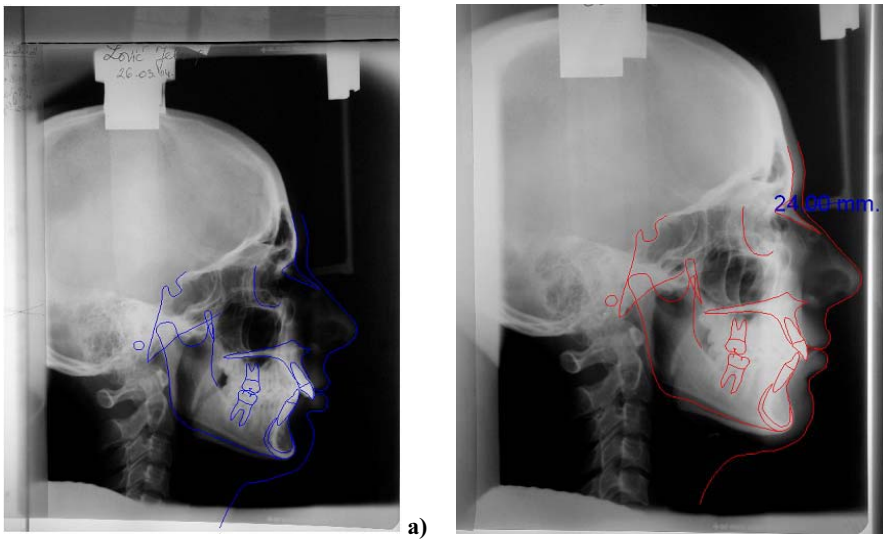
SO analysis	Variable (measurements to RLp in mm)	Before	After	After – Before (D)	Correction Maxilla+ Mandible
Skeletal + Dental	ms	64.35 ± 2.31	62.37 ± 2.27	1.98 ± 2.40	Molar relation
	mi	62.05 ± 2.51	64.38 ± 2.35	2.33 ± 0.73	4.31 ± 0.52
Skeletal + Dental	is	94.45 ± 2.81	91.41 ± 2.72	3.04 ± 1.35	Overjet
	ii	84.30 ± 2.65	88.23 ± 2.81	3.93 ± 1.32	6.97 ± 1.40
Skeletal	ss	83.14 ± 2.26	82.36 ± 2.07	0.78 ± 0.66	Skeletal correct.
	Pg	87.40 ± 2.86	88.44 ± 2.43	1.04 ± 1.21	1.82 ± 0.89
Dental (molars)	ms(D)-ss(D)	–	–	1.20 ± 0.51	Molars
	mi(D)-Pg(D)	–	–	1.29 ± 0.94	2.49 ± 0.87
Dental (incisors)	is(D)-ss(D)	–	–	2.26 ± 1.06	Incisors
	ii(D)-Pg(D)	–	–	2.89 ± 1.04	5.15 ± 1.83

RLp – occlusal reference line perpendicular; ms – the most mesial point of the approximal surface of the first upper molar; mi – the most mesial point of the approximal surface of the lower first molar; is – incisal edge of the upper incisor; ii – incisal edge of the lower incisor; ss – the most recessed point of the anterior side of the maxilla; Pg – the most prominent point of the skeletal chin profile.



a) **b)**

Fig. 5 – Computer drawings of anatomical details superimposition over the patient's photo profile: a) before the treatment; b) after the treatment.



a) **b)**

Fig. 6 – Profile cephalogram with computer-drown anatomical details: a) before treatment; b) after the treatment.

was almost 7 mm. The point ss changed its position slightly backward, while the point Pg changed its position anteriorly. Skeletal correction during the Herbst treatment was almost 2 mm.

Discussion

Advances in imaging are likely to enhance the accuracy and reliability of orthodontic diagnosis and treatment planning, and will be of importance in both clinical practice and research [10].

Herbst appliance therapy stimulates the growth of the mandible both by increasing the gonial angle and the growth of the condyle [11–15]. Skeletal and soft tissue facial profile convexity was reduced in adults and adolescents as a result of treatment by the Herbst appliance [16].

Examining the effects of the Herbst appliance in young adults with class II, division 1 malocclusion, Ruf and Pancherz [16] found that the same soft tissue changes occurred as in Herbst treatment in adolescent patients. The improvement of facial profile is combined with the upper lip retrusion but not with changes in the lower lip position. They state that both lips are supported by the upper incisors, thus significantly participating in the anteroposterior position of the upper lip in particular, while the lower incisors have no significant effect on the position of the lower lip. Flores-Mir et al. [6] found no difference in the effect of Herbst treatment on the soft tissue profile between adolescents and young adults, too. However, they point out that facial esthetic is generally improved and more detailed assessment of facial esthetics should be made after the examination of the patient's front face during conversation, function, individual facial expression and smile.

During Herbst appliance therapy anterior movement of the lower jaw occurs and the supramentale point also moves forward. The results obtained by this study show a change in slope of the Juanita line, which then rotates around the subnasal point. This is the reason for the surfaces of the soft tissues beyond the subnasal point to cover less of the reference space following the therapy, while the surfaces above this point cover more space. The movement of the lower jaw also brings about a forward shift of the pogonion point, so that the E line rotates around the tip of the nose. This change increases the surface of the free space within the reference space. Former studies based on profile cephalograms analysis of the same patients, show skeletal and dentoalveolar changes that supporting facial soft tissue change too [17–19]. Skeletal and dentoalveolar treatment changes in this study indicate the essential details. Upper incisors retrusion, lower incisor protrusion and forward movement of the lower jaw affect overjet reduction. It is the most important consequence to the correction and straightening of the lower facial third soft tissue.

Changes produced by fixed functional appliances seem to restrict forward movement of the upper lip [6]. A relative surface reduction of the upper lip is the consequence of a number of factors. Most patients targeted for Herbst appliance therapy have class II malocclusions, hence the reduction in the surface of the upper lip is caused by upper incisors, retrusion as well as maxillar growth inhibition [11, 12, 20]. In most cases, SNA angle reduction also occurs. Some authors cite that there is a reduction in upper lip prominence with no a change in inclination of the upper lip [21]. They explain this using similar type of movements of subnasale and labiale superior points. As explained earlier, Juanita line slope influences the reduction of the surface occupies by the upper lip. It is most probable that these changes are the result of a combination of skeletal and dentoalveolar changes [22–26].

Changes caused by fixed functional appliances seem to limit upper lip forward movement [6]. The relative surface of the upper lip increases due to lower lip forward movement. Mandibular advancement by the mandibular protraction appliance (MPA) similar to the Herbst appliance produces satisfactory results by reducing facial convexity. The treatment effect of this appliance is the correction of malocclusion through mesial displacement of the first mandibular molars, with the consequent protrusion of the lower lip [27]. The lower lip also moves forward because of lower lip position changes. As for the soft tissue, a significant forward movement of the pogonion (Pg′) was found in the treatment group compared with the controls. Our findings are supported by the authors who state that the fixed functional Jasper Jumper appliance (similar to the Herbst appliance) promotes horizontal growth at the pogonion area and that the overlying soft tissue reflects that change [28]. The increase in the lower lip surface reported in this study was not statistically significant, which can be explained by the slope of the Juanita line and by straightening of the inferiorly curved lower lip that is present with class II patients. According to Flores-Mir et al. [6] both lips are supported far more by the upper incisors and this is why protrusion of the lower incisors occurs during the therapy and is not followed by the expected protrusion of the lower lip. This has been also supported by other authors [22–26]. Thus, it can be concluded that the relation of soft tissues changes and skeletal structures is not linear, but a very complex one [29].

Even though the therapy caused the forward movement of the chin, its surface enclosed by the reference space was not reduced due to the simultaneous change in the slope of the Juanita line. The surface occupied by the nose within the reference field was increased due to Juanita line rotation.

The change in the slope of the Juanita and E lines caused changes in the proportions of the filled and free spaces of the soft tissue profiles. The presence of the free space was greater after the therapy, indicating improved aesthetics of the patient's profile. A remarkable improvement in the appearance of patient's profile was cited previously by other authors [11, 13, 20]. Even though Herbst appliance therapy could be followed by statistically significant changes in the soft tissues their clinical visibility is debateable. In order to conduct a detailed evaluation of the aesthetic effects of the therapy, an analysis of the appearance of the patient during different facial movements (*eg* smiling, different mimics, etc.) and during speech has to be performed. The use of stereophotogrametry and laser scans of the surface of the

face could overcome the limitations of the use of photogs for this purpose [6]. The use of the E-line during analysis of the soft tissue profile requires caution as simultaneous changes in soft tissue pogonion and pronasal occur and both have an effect on the appearance of the lips [6].

Skeletal, dental and soft-tissue changes induced by the Jasper Jumper appliance in late adolescence, that uses the E line as a reference line, were not found to produce significant antero-posterior changes of the upper lip [30]. However, statistically significant protrusion of the lower lip was noted which is contrary to the findings observed in the current study.

Conclusion

Based on the obtained results, soft tissue treatment effects of the Herbst appliance are: a reduction of the relative surface of the upper lip in both genders; a slight increase in the relative surface of the lower lip; a reduction of the relative surface occupied by the chin; a significant improvement of the profile appearance; a consequence of the upper incisors retrusion, lower incisor protrusion and forward movement of the lower jaw.

The extent of soft tissue changes that occur on its dentoalveolar and skeletal support is a complex issue.

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Received on June 22, 2011.
Revised on June 29, 2012.
Accepted on July 3, 2012.
OnLine-First June, 2013.

ORIGINAL ARTICLE

UDC: 616.89-008.441::616.31-052
DOI: 10.2298/VSP1401016J

Risk factors for severe dental anxiety among medical students

Faktori rizika od nastanka izrazitog straha od stomatološke intervencije kod studenata medicine

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Abstract

Background/Aim. Severe dental anxiety (SDA) is the most severe form of dental anxiety, thus the aim of this study was to determine the factors associated with SDA in students of health-related disciplines. **Methods.** In this case-control study the cases were students with severe dental anxiety. The study was conducted at the Faculty of Medical Sciences, University of Kragujevac, Serbia. The participants were undergraduate students attending lectures during spring semester 2010/2011 (n = 1,812). A random sample of 800 students was assessed for the association between various risk factors and the severe dental anxiety. The main outcome measures were the data on demographics, dental anxiety, habits concerning oral hygiene, nutrition, general anxiety and (co)morbidity which were collected from the study participants by semi-structured questionnaire. **Results.** Less frequent visits to the dentist (OR adjusted = 7.02 [2.65; 18.60]) and visiting the dentist only when there is a dental problem (OR adjusted = 8.08 [1.28; 50.93]) were associated with severe dental anxiety. The same was true for improper oral hygiene (OR adjusted = 4.25 [1.16; 15.60]). Factors as changing toothbrush more frequently (OR adjusted = 0.33 [0.14; 0.76]) and having chronic disease (OR adjusted = 0.01 [0.00; 0.09]) were inversely associated with severe dental anxiety. The level of education of students was not associated with severe dental anxiety. **Conclusion.** Inappropriate oral hygiene, less frequent changes of a toothbrush and less frequent visits to the dentist are important risk factors for severe dental anxiety.

Key words:
dental anxiety; students, medical; oral hygiene; dental care.

Apstrakt

Uvod/Cilj. Izraženi strah od stomatoloških intervencija spada u najsnažniji vid straha od zubara, te je cilj ove studije bio da se otkriju faktori koji utiču na nastanak izrazitog straha od stomatološke intervencije kod studenata fakulteta zdravstvene stuke. **Metode.** Učesnici u istraživanju bili su dodiplomski studenti Fakulteta medicinskih nauka Univerziteta u Kragujevcu, koji su pohađali nastavu tokom letnjeg semestra školske 2010/2011. godine (n = 1812). Metodom slučajnog uzorkovanja određeno je 800 studenata kod kojih je utvrđivana povezanost različitih faktora rizika i nastanka izrazitog straha od stomatološke intervencije. Izabrana je studija slučaj – kontrola, pri čemu su slučajeve činili studenti koji imaju izrazit strah od stomatološke intervencije. Svi učesnici istraživanja popunili su specijalno sastavljeni polu-strukturisani upitnik iz koga su dobijeni glavni ishodi studije: demografski podaci, stepen straha od stomatološke intervencije, navike vezane za higijenu usne duplje, način ishrane, stepen opšte anksioznosti i udružene bolesti. **Rezultati.** Ređi odlazak studenata kod stomatologa ($Or_{adjusted}$ = 7,02 [2,65; 18,60]) i odlazak samo kada su imali neki problem sa zubima ($Or_{adjusted}$ = 8,08 [1,28; 50,93]) bili su povezani sa izrazitim strahom od stomatološke intervencije. Isti rezultat je dobijen i kod studenata sa neadekvatnom higijenom usne duplje ($Or_{adjusted}$ = 4,25 [1,16; 15,60]). Ređi nastanak izrazitog straha od stomatološke intervencije bio je povezan sa češćim menjanjem četkice za zube ($Or_{adjusted}$ = 0,33[0,14; 0,76]) i hroničnim oboljenjem ($Or_{adjusted}$ = 0,01 [0,00; 0,09]). Stepen obrazovanja studenta nije bio povezan sa izrazitim strahom od stomatološke intervencije. **Zaključak.** Za nastanak izrazitog straha od stomatološke intervencije značajni faktori rizika su neadekvatna higijena usne duplje, ređe menjanje četkice za zube i ređi odlazak kod stomatologa na kontrolne preglede.

Ključne reči:
anksioznost, stomatološka; studenti medicine; usta, higijena; zubi, nega i lečenje.

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Introduction

Dental anxiety is exaggerated, fearsome psychological reaction of a person to possible or immediate dental intervention which seems to that person as being harmful or dangerous. Severe dental anxiety (SDA) is the most severe form of dental anxiety [1]. The prevalence of SDA is relatively high: in a study on urban population of Goteborg, Sweden, SDA was encountered in 6.7% when scale of dental anxiety with 10 questions was used, and in 5.4% with Corah's dental anxiety scale [1]. It is about four times higher in persons between 18 and 39 years of age than in persons older than 60 [2]. In Norway SDA was found among 11.3% of 25-year-old males and 19.8% of females of the same age, showing that it affects mostly younger people [3]. Severe dental anxiety has negative impact on dental and oral cavity health. In patients with SDA the average number of Decayed, Missing and Filled teeth (DMF) score is 18.6 ± 5.6, and deterioration of dental and oral cavity health, expressed as the presence of root remnants, could be found in as much as 57% of cases [4].

There are many factors potentially associated with SDA, but their absolute and relative importance was differently estimated in previous studies. While some of the studies found association of general anxiety with the SDA [2], other studies did not reach such conclusion [5]. Kirova et al. [6] found that age, level of education and type of labor correlate with the Corah's scale score, while sex of the patients did not. In a case/control study conducted on general population in Denmark, SDA was associated with fear of drilling, negative dentist contacts, general fear tendency, avoidance of treatment and increased oral symptoms [7]. Predictors of SDA in general French population were younger age, living on a farm and unskilled labor [8].

There are few published studies dealing with the prevalence and/or predictors of SDA in the population of university students. The study on 503 university students from various fields was conducted in Pakistan, showing that the prevalence of high to severe dental anxiety was 21.6% among males and 24% among females [9], much higher than in general population. Although there is little data on the prevalence among students of health-related disciplines, SDA in this subpopulation could adversely affect their attitude towards the patients with dental problems and aggravate their future communication with patients.

The aim of our study was to investigate the prevalence and risk factors associated with SDA in medical students.

Methods

Our study was conducted at the Faculty of Medical Sciences, University of Kragujevac, a higher public education institution situated at the town of Kragujevac, Serbia. The study population consisted of all the students of undergraduate studies who attended lectures regularly in spring semester of the school year 2010/2011 (n = 1.812).

The study was conducted on a random sample of 800 students in April and May, 2011. Demographic data and data on dental anxiety, habits concerning dental and oral cavity hygiene, nutrition, general anxiety and (co)morbidity were obtained from an anonymous structured questionnaire. All the data were obtained after previous written consent of the study participants, and the study protocol was approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac.

Based on the expected study power of 80%, the probability of the statistical error type 1 (α) of 0.05, two-way testing of difference in frequencies of risk factors between the study groups using $\chi 2$-test, and the expected difference of 12% in reasons for visiting a dentist (with frequency of 40% for a reason in one of the groups) we calculated a minimal sample size of 605 students in both groups [10]. In order to compensate for response failure, we decided to work with a sample of 800 students.

The study sample of 800 students was chosen randomly from the study population in following way: a list of all students was taken from the Faculty's offices and the students on the list were numbered from 1 to 1,820; then, using the function "randbetween" from the Microsoft Excel program, version 2007, 800 the students were chosen from the list.

The design of our study was of the case-control type, with the aim to assess the association between various risk factors and the occurrence of SDA. The study participants completed the Corah's dental anxiety self-rating scale. The Corah's dental anxiety scale consists of 4 items with score range from 4 to 20. Participants who score 15 or higher on the Corah's dental anxiety scale were considered to belong to the group with SDA. The cases were all students from the study sample with the SDA, i.e. having a score of 15 or higher on Corah's dental anxiety scale [1, 2]. The controls were selected from the study sample after matching with the cases by age and sex. From the matching students we selected four study participants for each case (sex- and age- matched), using the function "randbetween" from the Microsoft Excel program, version 2007. No exclusion criteria were employed for both cases and controls.

In order to identify potential risk factors, the following groups of variables were measured: socio-demographic data (sex, age, year of study, average grade, number of repeated academic years, monthly income of students' families and having a chronic disease), pattern of visiting dentist (frequency of visiting a dentist, reason for visiting a dentist, having a chosen dentist, using private or public dental service, having frightening experience with a dentist and family attitude towards visiting a dentist), pattern of keeping dental and oral cavity hygiene (frequency of brushing teeth, use of a dental floss, frequency of changing a toothbrush and average duration of brushing teeth), dental status (number of extracted permanent teeth, number of filled permanent teeth and number of decayed permanent teeth and halitosis) and feeding habits (frequency and quantity of sweets consumed and frequency and quantity of uncooked fruits and vegetables consumed). The variables were measured using structured questionnaire.

Dental anxiety was measured by the Corah's dental anxiety scale [1, 2]. General anxiety was measured by the Zung self-rating anxiety scale score. This scale consists of 20 self-

reported items related to anxiety symptoms with a total score range from 20 to 80. The score range 20–44 indicates normal state, 45–59 mild to moderate anxiety levels, 60–74 marked to severe anxiety levels and 75–80 extreme anxiety levels [11].

The prevalence of each risk factor was determined for both cases and controls. The differences between cases and controls in the observed characteristics were assessed by the Student *t*-test for continuous variables and the $\chi 2$-test for frequencies. The differences were considered significant if probability of null hypothesis was less than 0.05. In order to estimate the association between potential risk factors and SDA, crude and adjusted odds ratios (OR) with 95% confidence intervals (95% CI) were calculated using logistic regression [12, 13].

Results

The total number of students enrolled in the study was 800, out of who 620 students responded to the questionnaire (response rate 77.5%), and were included in final data analysis. Baseline characteristics of the cases and controls, and the differences between them, are shown in Table 1. The differ-

Table 1

Socio-demographic data, pattern of visiting the dentist, pattern of keeping dental and oral cavity hygiene, dental status and feeding habits in the study group and controls*

Variables	Study group (n = 46)	Controls (n = 184)	Statistical significance (*p*)	Crude OR (95% CI)
Sex, n (%)				
male	7 (15%)	28 (15)	1.000	1.00 (0.41, 2.46)
female	39 (85)	156 (85)		
Age (years), $\bar{x} \pm SD$	22.6 ± 3.7	22.1 ± 2.5	0.247	1.07 (0.96, 1.19)
Frequency of visiting a dentist, n (%)				
< once in 2 years	9 (20)	11 (6)	0.000**	2.21 (1.58, 3.09)
once in 2 years	11 (24)	17 (9)		
once per year	18 (39)	59 (32)		
≥ 2 times per year	8 (17)	97(53)		
Reason for visiting a dentist, n (%)				
primarily when there is a problem	41(89)	109(59)	0.000**	5.64 (2.13, 14.94)
primarily for check-up	5 (11)	75 (41)		
Number of decayed permanent teeth, $\bar{x} \pm SD$	1.8 ± 1.5	1.0 ± 2.5	0.030**	1.15 (0.97, 1.36)
Halitosis, n (%)	1/34/11	10/156/18		
yes	1 (2)	10 (5)	0.028**	2.70 (1.26, 5.82)
no	34 (74)	156 (85)		
do not know	11 (24)	18 (10)		
Frequency of brushing teeth times per day, $\bar{x} \pm SD$	2.4 ± 0.8	2.7 ± 0.8	0.013**	0.56 (0.35, 0.89)
Frequency of changing a toothbrush, n (%)				
once per 2 months or more frequently	21 (46)	65 (35)	0.214	0.95 (0.62, 1.46)
every 2–6 months	17(37)	99 (54)		
every 6–12 months	6 (13)	13 (7)		
once per year or more rarely	2 (4)	7 (4)		
Average duration of brushing teeth (minutes), $\bar{x} \pm SD$	2.8 ± 1.0	3.2 ±1.4	0.090	0.77 (0.57, 1.04)
Frequency of fresh fruits intake, n (%)				
every day	19 (41)	114 (62)	0.002**	1.79 (1.29, 2.46)
3–4 times per week	12 (26)	47 (26)		
once per week	6 (13)	15 (8)		
rarely	9 (20)	8 (4)		
Frequency of fresh vegetables intake, n (%)				
every day	19 (41)	114 (62)	0.006**	1.62 (1.18, 2.23)
3–4 times per week	12 (26)	47 (26)		
once per week	6 (13)	15 (8)		
rarely	9 (20)	8 (4)		
Having a chronic disease, n (%)				
yes	1 (2)	29 (16)	0.014**	0.12 (0.02, 0.90)
no	45 (98)	155 (84)		
Having frightening experience with a dentist, n (%)				
yes	23(50)	45 (24)	0.001**	3.09 (1.58, 6.03)
no	23(50)	139 (76)		
Zung Self-Rating Anxiety Scale score, $\bar{x} \pm SD$	34.7 ± 9.2	33.5 ± 8.6	0.421	1.02 (0.98, 1.05)

***For the sake of clarity, variables with frequency of an event less than 2% and some less important variables with insignificant differences between the cases and the controls are not shown in the table; **Significant difference; OR – odds ratios; CI – confidence interval.**

ences between the cases and controls were not significant in terms of age, sex, year of study, average grade, number of repeated academic years, monthly income of students families, frequency of visiting a dentist, number of extracted permanent teeth, number of filled permanent teeth, use of a dental floss, frequency of changing a toothbrush, average duration of brushing teeth, using private or public dental service, frequency and quantity of sweets consumed, quantity of uncooked fruits and vegetables consumed, family attitude towards visiting a dentist and the Zung self-rating anxiety scale score.

However, significant differences between the cases and controls were found in: the reason for visiting a dentist, the number of decayed permanent teeth, halitosis, frequency of brushing teeth, having a chosen dentist, frequency of uncooked fruits and vegetables consumed, having a chronic disease and having frightening experience with a dentist.

The results of the logistic regression analysis (Cox & Snell R square 0.405, Nagelkerke R square 0.633, Hosmer-Lemeshow Chi square 21.05, df = 8, $p = 0.007$) with adjustment for potential confounders are shown in Table 2. The (see Tables 1 and 2), after adjustment confidence limits of these odds ratios included the value of one.

The interaction between risk factors which are likely to have an additive risk for SDA was investigated (Table 3). The analysis did not show a clear synergistic effect for any of factors that were individually associated with SDA. Although adjusted odds ratios were still significant and their confidence limits excluded the value of one when the frequency of visiting a dentist interacted with the reason for visiting a dentist, or when the reason for visiting a dentist interacted with halitosis, synergistic effects could not be confirmed because adjusted odds ratios did not increase, but remained closer to one than odds ratios for individual factors.

Discussion

The obtained results show that the students visiting a dentist less frequently and only when they have a dental problem are more prone to have SDA. The same is true for those with improper oral hygiene and having halitosis. On the other hand, the study participants changing toothbrush

Table 2

Crude and adjusted odds ratios (OR) of the risk factors for severe dental anxiety

Risk factors	Crude OR (95% CI)	Adjusted* OR (95% CI)
Frequency of visiting a dentist (< once in 2 years/once in 2 years/ once per year/≥ 2 times per year)	2.21 (1.58, 3.09)	7.02 (2.65, 18.60)
Reason for visiting a dentist (primarily when there is a problem/primarily for check-up)	5.64 (2.13, 14.94)	8.08 (1.28, 50.93)
Halitosis	2.70 (1.26, 5.82)	4.25 (1.16, 15.60)
Frequency of changing a toothbrush (once per 2 months or more frequently/every 2-6 months/every 6-12 months/once per year or more rarely)	0.95 (0.62, 1.46)	0.33 (0.14, 0.76)
Having a chronic disease	0.12 (0.02, 0.90)	0.01 (0.00, 0.09)
Study course: physician/dentist/ pharmacist/nurse/physiotherapist	1.54 (1.22, 1.94)	1.53 (0.93, 2.51)
Frequency of brushing teeth (times per day)	0.56 (0.35, 0.89)	0.46 (0.19, 1.07)
Having frightening experience with a dentist	3.09 (1.58, 6.03)	6.35 (0.56, 73.07)
Having a chosen dentist	0.50 (0.26, 0.95)	2.60 (0.59, 11.38)

***Adjusted for year of study†, average grade†, number of repeated academic years†, monthly income of a student's family†, frequency of visiting a dentist†, number of extracted permanent teeth†, number of filled permanent teeth†, use of a dental floss†, frequency of changing a toothbrush, average duration of brushing teeth†, using private or public dental service†, frequency and quantity of sweets consumed†, quantity of uncooked fruits and vegetables consumed†, parental education†, family attitude towards visiting a dentist†, area of residence†, parental age†, number of siblings†, Zung Self-Rating Anxiety Scale score†, study course, reason for visiting a dentist, number of decayed permanent teeth†, halitosis, frequency of brushing teeth, having a chosen dentist, frequency of uncooked fruits and vegetables consumed†, having a chronic disease and having frightening experience with a dentist.**

†Crude and adjusted odds ratios are not shown in the table for the sake of clarity.

only significant associations were between severe dental anxiety and frequency of visiting a dentist ($OR_{adjusted}$ 7.02; CI 2.65, 18.60; $p = 0.000$), reason for visiting a dentist ($OR_{adjusted}$ 8.08; CI 1.28, 50.93; $p = 0.026$), halitosis ($OR_{adjusted}$ 4.25; 1.16, 15.60; $p = 0.029$), frequency of changing a toothbrush ($OR_{adjusted}$ 0.33; CI 0.14, 0.76; $p = 0.009$) and having a chronic disease ($OR_{adjusted}$ 0.01; CI 0.00, 0.09; $p = 0.002$). Although the crude odds ratios for frequency of brushing teeth, having frightening experience with a dentist and having a chosen dentist were significantly different from one more frequently and having chronic disease also less frequently have SDA. Our results are partially in accordance with the results of a Danish study [7], since visiting a dentist rarely and only when there is a problem is actually avoidance of treatment. The association of avoidant behavior, poor oral hygiene and the SDA was also observed in several case reports [14]. It is interesting that the level of education of the students (year of study) was not associated with the occurrence of SDA, showing that only understanding of the problem is not helpful for relieving SDA.

Table 3

Interactions between the frequency of visiting a dentist and the reason for visiting a dentist and the reason for visiting a dentist and halitosis

Variables	Crude odds ratio (95% CI)	Adjusted* odds ratio (95% CI)
No difference in frequency of visiting dentist	1.0 (reference)	1.0 (reference)
Only frequency of visiting a dentist (<once in 2 years/once in 2 years/ once per year/≥ 2 times per year)	2.21 (1.58, 3.09)	7.02 (2.65, 18.60)
Only reason for visiting a dentist (primarily when there is a problem/primarily for check-up)	5.64 (2.13, 14.94)	8.08 (1.28, 50.93)
Both frequency of visiting a dentist and reason for visiting a dentist	2.22 (1.65, 2.97)	4.87 (2.32, 10.20)
No halitosis	1.0 (reference)	1.0 (reference)
Only reason for visiting a dentist (primarily when there is a problem/primarily for check-up)	5.64 (2.13, 14.94)	8.08 (1.28, 50.93)
Only halitosis	2.70 (1.26, 5.82)	4.25 (1.16, 15.60)
Both reason for visiting a dentist and halitosis	2.21 (1.49, 3.28)	2.77 (1.31, 5.87)

***Adjusted for year of study, average grade, number of repeated academic years, monthly income of a student's family, frequency of visiting a dentist, number of extracted permanent teeth†, number of filled permanent teeth, use of a dental floss, frequency of changing a toothbrush, average duration of brushing teeth†, using private or public dental service, frequency and quantity of sweets consumed, quantity of uncooked fruits and vegetables consumed, parental education, family attitude towards visiting a dentist, area of residence, parental age, number of siblings, Zung Self-Rating Anxiety Scale score, study course, reason for visiting a dentist, number of decayed permanent teeth, halitosis, frequency of brushing teeth, having a chosen dentist, frequency of uncooked fruits and vegetables consumed, having a chronic disease and having frightening experience with a dentist.**

Inspite of widespread beliefs that fear and anxiety of dental treatment is a simple continuum of frightening experience that occurred in the past, this link is not straightforward, and there are numerous examples where previous frightening experience does not exist in persons with very SDA [15]. Whether a person will develop SDA after a painful experience with a dentist depends a lot on her/his cognitive abilities; it was shown that younger and less educated more frequently develop SDA than others [2, 3, 16]. Besides, good verbal and nonverbal communication between dentist and patient, which reflects care, respect and empathy, is strong preventative factor against the development of SDA [17].

An interesting finding in our study was that the students with chronic diseases had fewer chances to develop SDA. This could be explained by observation that children with chronic illness use coping as their predominant strategy for adapting to common painful and stressful events [18], which helps them to avoid developing SDA.

Frequency of brushing teeth was associated with decreased chances of the SDA in our study. Although there are no other studies confirming such link, frequent teeth brushing and other positive elements of oral hygiene behavior were linked to good dental caries status in students [19] and avoidance of visiting a dentist was associated with poor oral hygiene [20], suggesting an indirect association advocating our results.

The factors associated with SDA in our study were not interacting with each other, but acted rather independently. When interactions were introduced in the logistic regression model, the odds ratios for the associated factors decreased instead to increase, confirming their independence.

Although in children aged 3–6 years [21] and 15-year-olds [22] SDA is positively correlated with general anxiety, our study shows that this is not the case in student population. Our findings are in accordance with other studies of dental anxiety among patients older than 15 years, which either show that the correlation between SDA and general anxiety observed in children weakens with their transition to adolescence and adulthood [23], or do not find any correlation between SDA and general anxiety [24]. Previously published explanations of this phenomenon are only tentative, and further research is necessary for full understanding of this disagreement.

In our study we did not take into account variables representative of personality characteristics, which is serious limitation. It has already been shown in general population that neurotic extraverts who seek for novelty and experience brief dissociative periods and magical thinking are prone to develop SDA [25]. Perfectionism was found to be associated with general anxiety and distress in university students of health-related disciplines, and it might be associated with the SDA, as well [26]. Therefore, in order to better understand causes of SDA, future research of risk factors for SDA in medical students which will include personality traits is necessary.

Conclusion

The results of our study suggest that avoidant behavior in regard to visiting the dentist and poor oral hygiene are factors more frequently associated with SDA than patient's medical knowledge or previous negative experiences with the dentist.

Acknowledgements

This study was partially financed by the grant No. 175007 given by the Serbian Ministry of Education, Science and Technological Development and by the grant given by the Ministry of Science, Montenegro.

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Received on February 29, 2012.
Revised on October 1, 2012.
Accepted on October 15, 2012.

ORIGINAL ARTICLE

UDC: 616.22-006-083.85
DOI: 10.2298/VSP1401022M

Comparative videostroboscopic analysis after different external partial laryngectomies

Komparativna videostroboskopska analiza nakon parcijalnih laringektomija

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Abstract

Background/Aim. After external partial laryngectomias, videostroborscopy is very usefull in evaluation of postoperative phonatory mehanisms showing the "slow motion" of the vibrations of the remaining laryngeal structures. The aim of this paper was to compare the videostroboscopic characteristics of the vibration and to establish the differences in the phonation mechanisms depending on the type of external partial laryngectomy performed. **Methods.** This prospective study was conducted during the period 2003–2009 at the Ear, Nose and Throat Clinic, Clinical Center of Vojvodina, Novi Sad, including 99 patients with laryngeal carcinoma, treated with open surgical approach using different types of vertical and horizontal partial laryngectomy. Videostroboscopy was used to analyse vibrations of the remaining laryngeal structures. **Results.** The dominant vibration structure after partial horizontal laryngectomy, chordectomy, frontolateral laryngectomy and three quarter laryngectomy was the remaining vocal fold, after hemilaryngectomy it was the false vocal fold and after subtotal and near total laryngectomy it was the arythenoid. In patients with supracricoid hemilaryngopharyngectomy performed, many different structures were involved in the vibration. After most of the partial laryngectomies, vibrations can be found in the reconstructed part of the defect. In both horizontal and vertical partial laryngectomies movements of the larynx during phonation were mostly medial, while in cricohyoidoglottopexies they were anterior-posterior. Most of the operated patients (72.7%) had insufficient occlusion of the neoglottis during the phonation. **Conclusion.** Videostroboscopy is a useful method in examining the phonation mechanisms of reconstructed laryngeal structures after partial laryngectomy as well as in planning postoperative voice therapy.

Key words:
laryngectomy; treatment outcome; vocal cords; stroboscopy; diagnostic technics and procedures; laryngeal neoplasms.

Apstrakt

Uvod/Cilj. Nakon eksternih parcijalnih laringektomija, videostroboskopija je veoma korisna za procenu postoperativnog mehanizma fonacije pokazivanjem usporenosti vibracija preostalih laringealnih struktura. Cilj rada bio je da se međusobno uporede videostroboskopske karakteristike vibracija preostalih struktura larinksa i utvrde razlike fonacijskih mehanizama nakon različitih parcijalnih laringektomija otvorenim spoljašnjim pristupom. **Metode.** Ispitivanje je sprovedeno kao prospektivna klinička studija u periodu od 2003. do 2009. na Klinici za bolesti uva, grla i nosa, Kliničkog centra Vojvodine u Novom Sadu, a obuhvatilo je 99 bolesnika operisanih zbog karcinoma larinksa nekom od parcijalnih laringektomija, kod kojih je načinjena videostroboskopska analiza vibracija preostalih struktura i fonacijskih mehanizama. **Rezultati.** Dominantno mesto vibracije bila je glasnica kod horizontalne supraglotične laringektomije, hordektomije, frontolateralne laringektomije i kod tročetvrtinskih operacija. Ventrikularni nabor dominantno je vibrirao kod hemilaringektomije, a aritenoid kod suptotalnih i kod skoro totalne laringektomije. Kod suprakrikoidne hemilaringofaringektomije zabeleženo je učešće u vibraciji više različitih struktura. Moguće su vibracije i u zoni rekonstrukcije defekta kod većine operativnih tehnika. Kod horizontalnih i vertikalnih laringektomija pokreti larinksa u fonaciji bili su najčešće medijalni, dok su kod krikohioidoepiglotopeksije bili anterioposteriorni. Insuficijentna okluzija neolarinksa u fonaciji bila je prisutna kod većine operisanih bolesnika. (72,7%). **Zaključak.** Videostroboskopija je korisna metoda proučavanja fonacijskih mehanizama rekonstruisanog larinksa posle parcijalnih laringektomija, kao i kod planiranja vokalne terapije.

Ključne reči:
laringektomija; lečenje, ishod; glasne žice; stroboskopija; dijagnostičke tehnike i procedure; larinks, neoplazme.

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Introduction

External partial laryngectomies, horizontal and vertical ones, are a group of open approach preservative techniques for laryngeal cancer surgery. These operations are not only oncologically radical, but also provide good functional results in regard to breathing, swallowing and speech, due to the reconstruction of remaining laryngeal tissues. The ability to speech and communicate in everyday and professional life has a great influence on the quality of life of operated patients [1–3].

Videostroboscopy is very usefull in evaluation of postoperative phonatory mechanisms showing the "slow motion" of the vibrations of the remaining laryngeal structures. The site and the mechanism of the remaining mucosa vibrations are the key factors affecting the quality of speech following partial laryngectomy [4, 5].

The most preservative open technique for laryngeal cancer surgery is chordectomy. It includes the resection of the vocal fold, anteriorly from the anterior commisure, posteriorly to the vocal process of the arytenoid, lateraly including the internal perichondrium of the thyroid lamina. Defect following resection can be left to heal without reconstruction or it is reconstructed immediately using surrounding tissue, usually mucosa of the false vocal fold. After hemilaryngectomy (laterovertical laryngectomy), the vocal fold, up to the anterior commissure, is resected extending posteriorly to include a part of the arytenoid if necessary, together with Morgagni's ventricle and a false vocal fold with underlying thyroid cartilage [6]. The external perichondrium of thyroid lamina is usually used for reconstruction, as well as pyriform sinus mucosa and fascia of surrounding muscles, usually of sternohyoid one. Frontolateral (anterovertical) laryngectomy is a larger resection including also resection of the anterior commissure, anterior third of the contralateral vocal fold with underlying cartilage as well. Reconstruction is similar to the one following hemilaryngectomy. In horizontal supraglottic laryngectomy, depending on the extention of the carcinoma, the epiglottis, preepiglottic space and a part of the thyroid cartilage above the glottis are resected. Reconstruction includes suturing of the remaining thyroid cartilage to the hyoid bone and/or the base of the tongue [7]. Basic surgical principle of supracricoid partial laryngectomy with cricohyoidoepiglottopexy (CHEP) is in removal of the entire thyroid cartilage with both true vocal folds, both false vocal folds, both paraglottic spaces with preservation of cricoartytenoid unit which includes cricoid cartilage, at least one arytenoid cartilage, cricoarytenoid joint, posterior and lateral cricoarytenoid muscle together with ipsilateral recurrent and superior laryngeal nerve. Reconstruction is performed by suturing the cricoid to the epiglottis and hyoid bone [8]. Near total laryngectomy is a complex surgical procedure [9] with preservation of small part of the cricoid, one arytenoid, cricoarytenoid joint, one vocal fold and ipsilateral recurrent laryngeal nerve. These structures with overlying mucosa form a tunneled mucosal shunt between the trachea and pharynx that is controlled by remaining intrinsic laryngeal musculature with its nerve supply. This shunt forms a phonatory valve which is insufficient for breathing and therefore requires a permanent tracheostoma. Supracricoid hemilaryngopharyngectomy (SCHLP) is indicated in cases of pyriform sinus carcinoma. Resection includes ipsilateral structures above the cricoid without the external perichondrium of the thyroid cartilage which is used for reconstruction. The superior part of the defect is reconstructed with surrounding pharyngeal and arytenoid mucosa [10]. Three-quarter laryngectomy is regarded as supraglottic laryngectomy extended on one side to the glottic plane. Reconstruction of a new fold on the hemilaryngectomy side is through a triangular strip of the exterior thyroid perichondrium.

Since rare comparative studies can be found in the literature, the aim of this paper was to present and compare the videostroboscopic characteristics of vibration and to establish the differences in the phonation mechanisms depending on the type of partial laryngectomy performed.

Methods

This prospective clinical study during the period 2003–2009 at the University Ear, Nose and Throat Clinic in Novi Sad, Serbia, included 99 patients with open surgery for laryngeal cancer as primary therapy. The patients with recurrent or residual disease were not included in the study. The patients underwent different types of vertical and horizontal partial laryngectomy depending on the site and spread of the tumor. After local wound healing, postoperative swallowing rehabilitation, with sufficient breathing, with or without tracheostoma, depending on the type of laryngectomy, the patients were referred to the phoniatrician. Each patient underwent videostroboscopic examination using the videostroboscopic system Storz Pulsar Model 20 140020-2002 with a SONY video screen. After recording to a compact disc, findings were analyzed, frame by frame. The unique protocol was used in videostroboscopic analysis of the next parameters: the presence of vibrations of the remaining parts of the larynx Švocal fold, false vocal fold, arytenoid, epiglottis, base of tongue, pharyngeal mucosa (present-absent)Ć the presence of vibrations in the reconstructed region of the postoperative defect (present-absent); the types of the laryngeal movement during phonation (medially, anteriorly, posteriorly, anteroposteriorly); the level of approximation of the vibrating structures during phonation; level of neophonation (glottic, ventricular, chordo/ventricular, vestibular, subglottic); closure of neoglottis during the closed phase of vibration (present/absent).

The study was conducted after Ethics Committees approval and with each patient consent.

Results

Among 99 patients, there were 90 male and 9 female patients, the age range 40–80 years (80% of the patients being in the age group 50–70 years), 92 (93%) of the patients were cigarette smokers, while alcohol consumption was present in 85 (86%) patients. The next types of partial laryngectomy were performed: horizontal supraglottic laryngec-

tomy in 16 (16%), chordectomy in 22 (22%), hemilaryngectomy in 28 (28.5%), frontolateral laryngectomy in 19 (19.5%), CHEP in 5 (5%), three-quarter laryngectomy in 1 (1%), SCHLP in 4 (4%) and near-total laryngectomy in 4 (4%) patients.

Videostroboscopic examination postoperatively revealed that the dominant place of vibrations, out of all 99 patients, was the vocal fold – 75 (75.8%): in all 16 (100%) patients after partial horizontal supraglottic laryngectomy, in 20/22 (90.9%) patients after chordectomy, in 15/19 (78.9%) patients after frontolateral laryngectomy, in 3/4 (75%) after near total laryngectomy, in 2/4 (50%) after SCHLP and 1/1 (100%) patient after three-quarter laryngectomy performed. Vibration of the false vocal fold was found in 41 patients (41.4%); after hemilaryngectomy in 25/28 (89.3%), after frontolateral laryngectomy in 12 (63.2%), after chordectomy in 12 (54.5%), while after other types of the laryngectomy performed it was found only in single cases. Vibration of the arytenoid was registerd in 39 (39.4%) of the patients; in 15 (53.6%) of the patients after hemilaryngectomy in all patients with CHEP and near total laryngectomy and in 2 patients after SCHLP. Vibration of the epiglottis was present in 11 (11.1%) patients; 4 patients after hemilaryngectomy, 3/5 after CHEP and in single cases after other operations. (Table 1).

Medial movement of the remaining larynx during phonation was seen in 64 (64.6%) patients. It was the dominant direction of the movement during phonation after hemilaryngectomy, frontolateral laryngectomy, partial horizontal supraglottic laryngectomy, chordectomy and three-quarter laryngectomy. Some of the types of anterior or anterior-posterior movements were found in 22 (22.2%) patients; all the patients after CHEP, in 8 patients after hemilaryngectomy, in 5 patients after chordectomy and in single cases after other types of laryngectomy. In the group of patients with the same type of surgery, different types of movement could be detected as seen in table 2. (Table 2).

In 43 (43.43%) of the patients, the most satisfactory type of approximation – the glottic one, was found. In 22 (22.2%) it was ventricular, in 19 (19.19%) vestibular, in 16 (16.2%) chordo-ventricular and in 4 (4%) sugblottic level of approximation was found. Glottic approximation was present in all the patients after partial horizotal supraglottic laryngectomy, while all the patients after CHEP had vestibular approximation. After hemilaryngectomy, all levels of approximation were present. After chordectomy, 11 (50%) patients had glottic, and other half had other different levels of approximation. After frontolateral laryngectomy, glottic approximation was found in the same number of patients – 8 (42%) as the ventricular one. After SCHEP, one half of the patients had vestibular and the other half had chordoventricular approximation.

Table 1

Vibrating structures in patients depending on the type of external partial laryngectomy performed

Types of partial laryngectomy	Number (%) of patient			
	Vocal fold	False vocal fold	Epiglottis	Arythenoid
Hemilaryngectomy	18 (64.3)	25 (89.3)	4 (14.3)	15 (53.6)
Frontolateral	15 (78.9)	12 (63.2)	1 (5.3)	8 (42.1)
PHSL	16 (100.0)	0 (0.0)	0 (0.0)	1 (6.3)
CHEP	0 (0.0)	0 (0.0)	3(60.0)	5 (100.0)
Three-quarter	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Near-total	3 (75.0)	0 (0.0)	0 (0.0)	4 (100.0)
Chordectomy	20 (90.9)	12 (54.5)	1 (4.5)	4 (18.2)
SCHLP	2 (50.0)	2 (50.0)	2 (50.0)	2 (50.0)
Total	75 (75.8)	41 (41.4)	11 (11.1)	39 (39.4)

PHSL – Partial horizontal supraglottic laryngectomy; CHEP – cricohyoidoepiglottopexy; SCHLP – supracricoid hemilaryngopharyngectomy.

Table 2

Movement of the remaining laryngeal structures in patients during phonation

Types of partial laryngectomy	Number (%) of patient						
	A	B	C	D	E	F	G
Hemilaryngectomy	5 (17.9)	0 (0.0)	3 (10.7)	7 (25.0)	8 (28.6)	2 (7.1)	1 (3.6)
Frontolateral	2 (10.5)	0 (0.0)	0 (0.0)	8 (42.1)	7 (36.8)	0 (0.0)	2 (10.5)
PHSL	1 (6.3)	0 (0.0)	0 (0.0)	4 (25.0)	3 (18.8)	0 (0.0)	0 (0.0)
CHEP	1 (20.0)	0 (0.0)	4 (80.0)	1 (20.0)	0 (0.0)	1 (20.0)	0 (0.0)
Three-quarter	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
Near-total	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chordectomy	1 (4.5)	0 (0.0)	4 (18.2)	13 (59.1)	11 (50.0)	0 (0.0)	1 (4.5)
SCHLP	0 (0.0)	0 (0.0)	1 (25.0)	1 (25.0)	0 (0.0)	1 (25.0)	0 (0.0)
Total	10 (10.1)	0 (0.0)	12 (12.1)	34 (34.3)	30 (30.3)	4 (4.0)	4 (4.0)

Movement of laryngeal structures: A – anterior, B – posterior, C – anteroposterior, D – right-medial, E – left-medial, F – from right to the left side, G – from left to the right side; PHSL – partial horizontal supraglottic laryngectom; CHEP – cricohyoidoepiglottopexy; SCHLP – supracricoid hemilaryngopharyngectomy.

Vibrations in the region of reconstruction were found in 51 (54.8%) patients; in 17 (68%) after hemilaryngectomy, in 14 (77%) after frontolateral laryngectomy and in rare single cases after other laryngectomies.

In 27 (27.3%) operated patients, inspite the postoperative defect, satisfactory neolaryngeal occlusion was established. Other 72 (72.7%) had insufficient occlusion. The highest percentage of the patients with insufficient occlusion was found in the patients after hemilaryngectomy – 26 (92.9%), after chordectomy in 17 (77.3%), after frontolateral laryngectomy in 11 (57.9%), after horizontal partial supraglottic laryngectomy in 9 (56.3%), in 4/5 patients after CHEP, in 3/4 patients after SCHLP and in one patient after near total laryngectomy.

Discussion

Analysis showed that the dominant place of vibration is the true vocal fold after partial horizontal supraglottic laryngectomy, chordectomy, frontolateral laryngectomy and three-quarter laryngectomy. False vocal fold dominantly vibrates after hemilaryngectomy, while the arytenoid is the dominant vibrating structure after subtotal and near total laryngectomy. In partial pharyngolaryngectomies all the remaining structures equally participate in vibration. This study shows that the false vocal fold is the important substitutional source of phonation, especially after hemilaryngectomy. Due to its size, the false vocal fold can compensate the large postoperative defect after hemilaryngectomy and allow sufficient phonation and swallowing [11]. Granqvist i Lindestad, as cited by Kendall et al. [12], 2005, using ultra fast photography found covibrations of the false vocal folds in healthy individuals, with different vibration frequency comparing to that of the true vocal folds. It is to assume that in pathological conditions, when the true vocal fold vibrations are absent, these vibrations became the dominant vibrating mechanism. Vibrations in the zone of defect reconstruction, with irregular form, were recorded after all types of operation in 54.5% patients. In most of the cases, the mucosa was used in the defect reconstruction as potentionally vibrating structure. Movements of the neolaryngeal structures were dominantly medial after partial horizontal supraglottic laryngectomy, chordectomy, hemilaryngectomy, frontolateral laryngectomy and three-quarter laryngectomy. After these types of operation, the larynx preserves physiological movements of its remaining structures. After subtotal laryngectomies, the dominant direction of movements is anteroposterior since epiglottis and arytenoid are the only remaining moving structures. In cases when only one arytenoid is present, such as after CHEP, anteroposterior movement is dominant during which the arytenoid falls anteriorly making contact with its apical part with epiglottis where the contact vibration is made. In cases when both arytenoids are present, preserved interarytenoid muscle prevents the arytenoid to fall anteriorly, therefore adduction movements are the dominant ones. Good contact between the epiglottis and arytenoid with contact vibrations are the prerequisite for satisfactory voice function. The presence of one or both arytenoids does not affect the voice quality significantly [13], but, on the other hand, preservation of both arytenoid shortens the time of swallowing rehabilitation [14]. After SCHLP, all types of laryngeal movements during phonation were recorded. Contralateral movement (hypercompensation) was noticed in single cases in all types of partial laryngectomies. The level of approximation during occlusion was dominantly glottic after partial horizontal supraglottic laryngectomies, chordectomies and three-quarter laryngectomies. After frontolateral laryngectomies, glottic and ventricular level of approximation were equally present, while after hemilaryngectomy ventricular level was the dominant one. Vestibular approximation was recorded after CHEP and SCHLP. After near-total laryngectomy, the contact was recorded along the inferior edge of the preserved vocal fold. Most (72.7%) of the operated patients had insufficient occlusion of the neolarynx due to postoperative defect. In this study, after partial horizontal supraglottic laryngectomy, minimal glottic gap was found in more than half of the patients but with no signifficant affect on the voice quality since only the glottic gap bigger than 1 mm causes breathy voice according to Schneider and Bigenzahn [15].

Similar results, in regard to presence of vibrations, movement of the neolarynx during phonation, occlusion of the neolarynx after analysing results after every single type of partial laryngectomy, were found by other authors [16–21].

Conclusion

The dominant site of vibration after partial horizontal supraglottic laryngectomy, chordectomy, frontolateral laryngectomy and three-quarter laryngectomy is the preserved vocal fold. The false vocal fold is a dominantly vibrating structure after hemilaryngectomy, while after subtotal and near-total laryngectomy it is the arytenoid. After most of partial laryngectomies, vibrations can occur in the zone of the defect reconstruction. After partial horizontal supraglottic and vertical partial laryngectomies, movements of the larynx during phonation are mostly medial, while after subtotal laryngectomies these movements are anteroposterior. Vocal insufficiency of the neolarynx is present in most of the operated patients. Videostroboscopy is a usefull method in evaluation of the phonation mechanisms after different partial laryngectomies. Without compromising oncological radicality, whenever it is possible, the types of partial laryngectomies that provide approximation and vibration at the glottic level or the ones with the best possible neolarynx occlusion, should be performed. In order to improve the quality of life and proffesional activity, all the patients with postoperative moderate and severe hoarsness, should be reffered to the phoniatrician and speach therapist for examination and voice therapy. The aim of this early rehabilitation is to increase the strenght, endurance and flexibility of the remaining laryngeal structures and incourage development of new zones of vibration.

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Received on February, 11 2012.
Revised on November 8, 2012.
Accepted on November 13, 2012.

ORIGINAL ARTICLE

UDC: 613.25:616.39]:616.12-089-036.8
DOI: 10.2298/VSP1401027C

The impact of obesity on early mortality after coronary artery bypass grafting

Uticaj gojaznosti na rani mortalitet nakon koronarne *bypass* hirurgije

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Abstract

Background/Aim. It had been suggested that elevated body mass index (BMI) is a beneficial and preventive factor when it comes to the outcome for patients undergoing coronary artery bypass grafting (CABG). At the same time, obesity is strongly associated with coronary artery disease development. The aim of this study was to determine the significance of the obesity paradox in patients referred for CABG and to examine if a relationship exists between obesity and early coronary surgery outcome. **Methods.** This study comparised 791 patients who had undergone isolated CABG over one year period (year 2010). The average age of patients was 62.33 ± 8.12 years and involved 568 (71.8%) male and 223 (28.2%) female patients, while the mean logistic EuroSCORE was 3.42%. The patients were categorized into three distinct groups based on their BMI: I – BMI < 24.9 kg/m^2; II – BMI 25–30 kg/m^2; III – BMI > 30 kg/m^2. Regression analysis was conducted to determine whether BMI was an independent predictor of early mortality after CABG. **Results.** The majority of the cohort could be categorized as overweight (49%) or obese (30%). There was no association between BMI and gender (p = 0.398). The overall early mortality was 2.15% (1.85% in the group I, 2.06% in the group II and 2.51% in the group III; p = 0.869). Univariate analysis showed that obesity cannot be regarded as an independent risk factor for early mortality following CABG (odds ratio 1.021, 95% confidence interval 0.910–1.145, p = 0.724). Duration of in-hospital period following the surgery was comparable within the BMI groups (p = 0.502). **Conclusion.** Compared to non-obese patients, overweight and obese individuals have similar early mortality rate following CABG. This study can substantiate the presence of obesity paradox only in terms that elevated BMI patients have comparable outcome with non-obese. Further research is needed to delineate potential underlying mechanisms that set off obesity to protective factor for coronary surgery.

Key words:
obesity; body mass index; myocardial revascularization; postoperative period; mortality.

Apstrakt

Uvod/Cilj. U ranijim radovima nagovešteno je da povišen indeks telesne mase (ITM) može biti koristan i da se može smatrati preventivnim faktorom kada se radi o ishodu operacije kod bolesnika koji se podvrgavaju koronarnoj *bypass* hirurgiji. Istovremeno, gojaznost se dovodi u vezu sa nastankom i razvojem koronarne bolesti. Cilj ove studije bio je utvrđivanje značaja paradoksa gojaznosti kod bolesnika koji su upućeni na hirušku revaskularizaciju miokarda, kao i isptivanje uticaja gojaznosti na rani ishod koronarne hirurgije. **Metode.** Studijom je obuhvaćen 791 bolesnik podvrgnut izolovanoj koronarnoj hirurgiji tokom jednogodišnjeg perioda (2010. godina). Prosečno životno doba 568 (71,8%) bolesnika muškog pola i 223 (28,2%) bolesnika ženskog pola iznosilo je 62,33 ± 8,12 godina, dok je prosečna vrednost logističkog EuroSCORE iznosla 3,42%. Bolesnici su bili svrstani u tri grupe shodno njihovom ITM: I – ITM < 24,9 kg/m^2; II – ITM 25-30 kg/m^2; III – ITM > 30 kg/m^2. Regresiona analiza korišćena je kako bi se ispitalo da li je ITM nezavisni prediktor ranog mortaliteta nakon koronarne hirurgije. **Rezultati.** Većina bolesnika imala je prekomernu telesnu masu (49%) ili su bili gojazni (30%). Nije postojala povezanost između ITM i pola (p = 0,398). Prosečna rana smrtnost iznosila je 2,15% (1,85% u grupi I, 2,06% u grupi II i 2,51% u grupi III; p = 0,869). Univarijantna analiza pokazala je da se gojaznost ne može smatrati nezavisnim faktorom rizika od ranog mortaliteta nakon koronarne hirurgije (unakrsni odnos 1,021; 95% interval poverenja 0,910–1,145; p = 0,724). Dužina postoperativnog bolničkog perioda bila je uporediva među ITM grupama (p = 0,502). **Zaključak.** U poređenju sa negojaznim bolesnicima, bolesnici sa prekomernom telesnom masom i gojazni imaju sličnu stopu ranog mortaliteta nakon koronarne hirurgije. Ova studija može potvrditi prisustvo paradoksa gojaznosti samo u smislu da bolesnici sa povišenim ITM imaju uporediv ishod operacije sa negojaznim bolesnicima. Dalja istraživanja su neophodna kako bi se odredili mehanizmi koji utiču na to da gojaznost postane protektivni faktor za koronarnu hirurgiju.

Ključne reči:
gojaznost; telesna masa, indeks; miokard, revaskularizacija; postoperativni period; mortalitet.

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Introduction

Obesity has been increasing in epidemic proportions in both adults and children [1]. Obesity is commonly regarded as a risk factor in development of different forms of cardiovascular diseases (CVD) such as coronary artery disease (CAD), heart failure, stroke, endothelial dysfunction, systolic and diastolic dysfunction and atrial fibrillation [2, 3]. The prevalence of obesity is on the rise and consequently larger population of obese patients are considered for some form of revascularization procedure including coronary artery bypass graft (CABG) or percutaneous coronary intervention (PCI).

Although long-term longitudinal studies have associated obesity with excess rates of cardiovascular morbidity and mortality, limited data exist on links between coronary artery revascularization and obesity [4]. Because obesity is considered to be a risk factor for CAD development, a conclusion is often drawn that obese patients should have poorer outcomes than non-obese patients. Surprisingly, several recent studies suggested that higher body mass index (BMI) is of benefit when it comes to lowering the mortality rate after heart surgery [2, 3, 5, 6].

Obesity is usually defined through BMI that is easily calculated [7]. BMI's lack of ability to differentiate body fat and lean mass is sometimes viewed as its weakness in improving clinical outcome prediction for overweight and obese patients [8–10].

There is a considerable disagreement regarding the influence of obesity on prognosis after heart surgery, with some major systems of classification indicating that obesity is associated with the poor outcome [11–14]. Despite the positive correlation between higher BMI and CAD development, many studies suggest better outcome and prognosis in obese patients with CAD and in patients undergoing revascularization (PCI and CABG) [15–19]. The obesity paradox is a term that describes the favourable short and long-term results after CABG in overweight and obese patients. Romero-Corral et al. [18], in their systemic review comprised of more than a quarter of a million patients, described the association between lower total and cardiovascular mortality and BMI status of patients with CAD in the sense that overweight and obese patients have a reduced risk as compared to underweight and normal weight individuals. According to these studies, a dual nature of obesity is evident. On the other hand, several studies found that obese patients have a higher incidence of morbidity and mortality after CABG [15]. It has been shown that obese patients are referred to surgery at a younger age and, in addition, with a higher frequency of comorbidities such as hypertension and diabetes mellitus.

The underlying mechanism that could explain obesity paradox remains elusive. It has been speculated that several factors – hormonal, inflammation, nutritional, and metabolic – might be involved in developing protective effect in population with elevated BMI and CAD [20]. Adipose tissue is increasingly being recognized as an active endocrine organ; however, the effects of "adipokines" on coronary arteries and atherosclerosis are not fully understood and the exact nature of this association is yet to be defined.

The aim of this study was to determine the significance of the obesity paradox in patients referred for CABG and to examine if a relationship exists between obesity and early coronary surgery outcome.

Methods

This prospective study included all the patients submitted to isolated CABG during a year period. Patients undergoing additional procedures in conjunction with CABG were excluded from this study. The resulting cohort consisted of 791 patients. The hospital Ethical Committee approved the study protocol and waived the requirement of informed consent before the commencement of data acquisition.

The National Heart, Lung, and Blood Institute and the World Health Organization as mentioned have adopted a weight classification for BMI that is calculated by dividing a patient's weight in kilograms by that patient's height in meters squared [7]. According to this classification, a BMI < 18.5 kg/m^2 was considered underweight, a BMI of 18.5 to 24.9 kg/m^2 was considered normal, a BMI of 25 to 30 kg/m^2 was considered overweight, and a BMI > 30 kg/m^2 was considered obese. Since the number of patients with BMI of less than 18.5 kg/m^2 was too small and the fact that there was no mortality, this subgroup was appended to the 18.5–24.9 kg/m^2 BMI group creating unified < 25 kg/m^2 group. A patient's BMI was calculated in the same hospitalization as the operative treatment.

Several groups of risk factors were examined: demographic – age, sex; comorbidities – chronic obstructive pulmonary disease (COPD), peripheral vascular disease, previous stroke, unstable angina, recent myocardial infarction (0–90 days prior the surgery), ejection fraction of the left ventricle and diabetes mellitus; surgical parameters: logistic EuroSCORE [21], aortic cross-clamping time, extracorporeal circulation time, number of distal coronary anastomoses and number of postoperative days. Surgical outcome defined as early mortality included 30–day mortality or mortality during the same hospitalization as the operation (status of every patient was verified by telephone on the 30th postoperative day).

The results are reported as mean ± standard deviation (SD) for quantitative variables and are summarized by absolute frequencies and percentages for categorical variables. Assessments of the continuous variables were performed using one-way analysis of variance (ANOVA). The χ^2 analyses were used to assess categorical variables and outcomes the Mann-Whitney test was used for non-parametric comparison. Multivariate logistic regression models for assessing the relation between BMI (as the classified form) and early mortality rate and presence of confounders were established, and associations are expressed as odds ratios (OR) with 95% confidence intervals (CI). The significance level for all analyses was $\alpha = 0.05$. Statistical analysis was performed using SPSS 13.0 for Windows (SPSS, Chicago, IL).

Results

This study comparised 791 patients who had undergone isolated CABG over one year period (year 2010). The aver-

age age was 62.33 ± 8.12 years and involved 568 (71.8%) male and 223 (28.2%) female patients, while the mean logistic EuroSCORE was 3.42%.

Baseline patient characteristics and comorbidities are listed in Table 1. Of 791 patients, 629 (79.52%) were over-

and the lowest prevalence in the high BMI (≥ 30 kg/m^2) group] and renal failure [the highest prevalence in the low BMI (< 18.4 kg/m^2) group and the lowest prevalence in patients with BMI (≥ 25 kg/m^2)]. The patients with higher BMI tended to be younger and there was an overall trend

Table 1

Baseline patient characteristics, comorbidities and surgical parameters

Parameter	BMI (kg/m^2) (number of patients)				*p*
	≤ 18.4 (n = 7)	18.5–24.9 (n = 155)	25–29.9 (n = 390)	≥ 30 (n = 239)	
Age, years, (x̄ ± SD)	61.00 ± 7.09	63.63 ± 8.22	63.00 ± 7.88	60.46 ± 8.20	< 0.0001
Female, n (%)	3 (42.9)	44 (28.4)	101 (25.9)	75 (31.4)	0.398
COPD, n (%)	1 (14.3)	8 (5.2)	25 (6.4)	22 (9.2)	0.344
PVD, n (%)	4 (57.1)	43 (27.7)	91 (23.3)	39 (16.3)	0.005
Previous stroke, n (%)	0 (0%)	3 (1.9)	10 (2.6)	8 (3.3)	0.812
Serum creatinine >200 µmol/L, n (%)	1 (14.3)	2 (1.3)	1 (0.3)	1 (0.4)	<0.0001
Unstable angina, n (%)	1 (14.3)	42 (27.1)	86 (22.1)	54 (22.6)	0.578
Ejection fraction, n (%)	52.86 ± 12.95	51.42 ± 10.40	54.18 ± 8.70	53.09 ± 9.41	0.02
Recent MI, n (%)	3 (42.9)	35 (22.6)	69 (17.7)	49 (20.5)	0.233
Urgent surgery, n (%)	0 (0)	3 (1.9)	7 (1.8)	5 (2.1)	0.976
Logistc EuroSCORE, (x̄ ± SD)	3.39 ± 1.35	4.15 ± 5.08	3.28 ± 3.51	3.18 ± 4.33	0.107
IDDM, n (%)	0 (0)	19 (12.3)	32 (8.2)	27 (11.3)	0.317
NIDDM (%)	3 (42.9)	36 (23.2)	104 (26.7)	78 (32.6)	0.144
Ao-X (min), (x̄ ± SD)	64.57 ± 28.11	58.37 ± 23.53	56.13 ± 30.46	55.72 ± 24.93	0.666
ECC time (min), (x̄ ± SD)	80.86 ± 38.37	70.17 ± 27.57	67.36 ± 33.47	66.06 ± 26.25	0.379
No. of distal anastomoses, (x̄ ± SD)	2.71 ± 0.76	2.65 ± 0.93	2.63 ± 0.93	2.55 ± 0.82	0.630
Postoperative days, (x̄ ± SD)	8.29 ± 1.11	9.41 ± 4.94	10.03 ± 7.88	10.43 ± 7.03	0.502
30-day mortality, n (%)	0 (0)	3 (1.93)	8 (2.05)	6 (2.51)	0.869

BMI – body mass index, COPD – chronic obstructive pulmonary disease; PVD – peripheral vascular disease; MI – myocardial infarction; IDDM – insulin dependent diabetes mellitus; NIDDM – non-insulin dependent diabetes mellitus; Ao-X – aortic cross clamp; ECC – extracorporeal circulation.

weight or obese, 155 (19.60%) were of normal weight, and 7 (0.88%) underweight (Figure 1). There were more men than women in all the BMI groups. The prevalence of comorbidities was comparable between the BMI groups (p > 0.05) except for the peripheral vascular disease [the highest prevalence in the low BMI (< 18.4 kg/m^2) group

toward lower mean age through increasing BMI group. Obese patients are submitted to coronary surgery on average 3 years earlier than normal weight or overweight population (p < 0.0001).

Surgical parameters and early mortality are summarized in Table 1. The mean BMI of the patients with early mortality was 28.14 ± 4.04 as compared to the patients with no mortality 28.12 ± 4.10 (p = 0.629) (Figure 2). There was no significant difference in aortic cross-clamp time, extracorporeal time, number of distal coronary anastomoses and

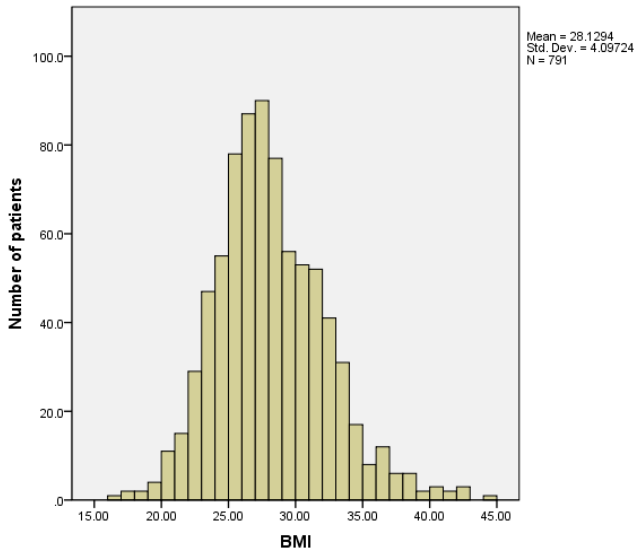

Fig. 1 – Number of patients in relation to a body mass index (BMI) value.

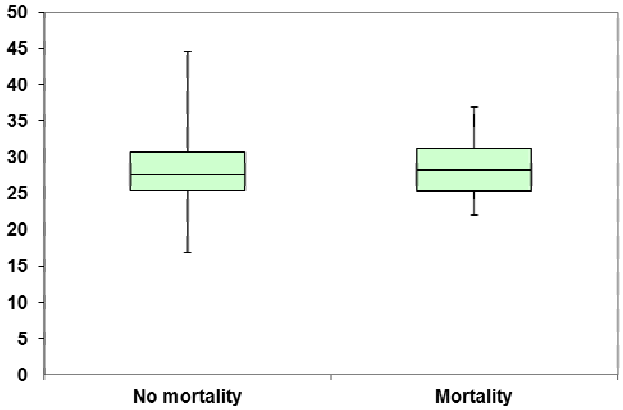

Fig. 2 – Box plot of the body mass index (BMI) values between the groups of patients in terms of mortality (p = 0.629).

number of postoperative days ($p > 0.05$). The average number of distal coronary anastomoses decreased as the value of BMI increased leading to decreased aortic cross-clamping time and subsequently extracorporeal time. This correlation did not reach the level of statistical significance. The average number of postoperative days increased in correlation with BMI value but, again, failed to reach the level of statistical significance ($p = 0.502$).

We conducted uni- and multivariate analyses to determine whether there was a relationship between early mortality and BMI. Regression analysis failed to designate BMI as an independent predictor of early mortality (OR = 1.021, 95% CI 0.910–1.145, $p = 0.724$). Table 2 demonstrates this relationship in univariate and multivariate analyses.

Table 2

Relationship between the 30-day mortality and BMI – univariate and multivariate analysis

BMI (kg/m^2)	Univariate analysis			Multivariate analysis*		
	OR	95% CI	*p*	OR	95% CI	*p*
≤ 24.9	Reference			Reference		
25–29.9	1.061	0.278–4.053	0.931	1.720	0.388–7.635	0.476
≥ 30	1.305	0.321–5.296	0.710	2.824	0.598–13.346	0.190

BMI – body mass index; OR – odds ratio; CI – confidence interval.
***Adjusted for age, peripheral vascular disease, serum creatinine > 200 μmol/L and ejection fraction.**

Discussion

There is a direct association between BMI and the incidence of CAD, however, once CAD is expressed, the association among BMI and prognosis becomes more complex [22]. Cardiac surgeons recognize obesity as a risk factor for perioperative adverse outcomes following CABG. Specifically, BMI greater than 30 are thought to be associated with excess of surgical risks [23]. Data regarding the influence of BMI on CABG outcome and prognosis are conflicting. Instead, increased incidence of complications such as sternal and superficial wound infection, saphenous vein harvest site infection and dysrhythmias was observed [24–26].

The results of this research indicate that obesity alone is not associated with early mortality after isolated CABG. The author's hypothesis that patients with an obese BMI are at greater risk of mortality after CABG was not substantiated. There is a strong relationship between the age of the patients undergoing CABG and the BMI value ($p < 0.0001$) in terms that the patients with high BMI are referred to CABG at younger age; a known effect of accelerated progression of atherosclerosis in obese population with clinical manifestation appearing a decade earlier than in normal weight population. It should be noted that for every 1% above ideal BMI values, the risk for CAD increases by 3.3% for women and by 3.6% for men [12]. The overall mortality for the entire group of our patients, regardless the BMI value, was 2.15% which is somewhat different from the other authors [17, 19, 24]. In the same time, the average BMI value of our patients was 28.13 kg/m^2 which is significantly lower than in other studies [26, 27]. These sample or population differences as well as the analytical approach and study design may give rise to different conclusions regarding the influence of BMI on early post-CABG mortality.

Several studies found that low BMI patients are at a higher risk [15, 27]. These studies showed that patients in the underweight group experienced greatest mortality and other complications after CABG. It was suggested that a low BMI might be a marker for multiple risk factors such as peripheral vascular disease, cerebrovascular disease, and smoking history. In our study, we had only 7 patients designated as underweight with no mortality in this group and, therefore, no clinical implications. A low BMI might also be a marker for more advanced heart disease [28].

Wigfield et al. [23] evaluated the effect of severe obesity (BMI > 40 kg/m^2) on the outcome of cardiac surgery and found that extreme obesity is not associated with an increased mortality after cardiac surgery. In our research, the number of patients with extreme BMI values (> 40 kg/m^2) was too small, only 9 of them, and we, also, did not find any association between high BMI values and early mortality. In contrast, Prabhakar et al. [29] used a very large database of 559,004 patients undergoing CABG to show an increased operative risk for moderately obese (BMI 35–39.9) and extremely obese (BMI > 40) patients (OR 1.21, 95% CI 1.1–1.3; OR 1.58, 95% CI 1.4–1.7, respectively).

In a propensity-matched analysis of 6,068 consecutive patients undergoing primary CABG at a single centre from 1991 to 2003, two propensity models were derived comparing all small patients with normal-sized persons and all obese patients with normal-sized persons [30]. Mortality was observed to be higher in very obese patients (BMI ≥ 36 kg/m^2) than in normal-sized individuals, but a worse survival rate was not observed among moderately obese patients (BMI 32–36 kg/m^2). This statement is in concordance with our results. Since most of our patients were categorized as overweight and moderately obese with a negligible percentage of severely and morbidly obese patients, BMI had no significant influence on early mortality following CABG.

The study by Reeves et al. [31] demonstrated that obese patients may be less likely to be selected for CABG and that, in general, obese patients undergoing CABG may have less high-risk features in particular less severe CAD and left ventricle dysfunction compared with lean patients, even when adjusting for these confounding factors. They demonstrated that underweight patients had a higher risk of perioperative complications, whereas the perioperative prognosis was not adversely affected by overweight and obesity.

Several studies tried to examine the effect of revascularization strategy and level of obesity on short- and long-term results. These attempts brought even more confusion in the field. Investigators from the Bypass Angioplasty Revascularization Investigation (BARI) trial had found that an increased BMI is associated with a worse long-term outcome after CABG but not after percutaneous coronary intervention (PCI) [32]. In contrast, the Arterial Revascularization Therapies Study (ARTS) which included a large cohort of patients who had multi-vessel CAD and underwent surgical or percutaneous revascularization, found that BMI had no effect on the 3-year outcome on those who underwent stenting [4]. "Conversely, among patients who underwent CABG, those who were overweight or obese had a significantly better outcome than did those who had a normal BMI with regard to survival without major adverse cardiac or cerebrovascular events, mainly due to lower rates of repeat revascularization procedures" [4].

A recent meta-analysis investigated the effect of obesity on short- and long-term mortality post-coronary revascularization [17]. The analysis included 22 cohort publications, reporting results in 10 post-PCI and 12 post-CABG populations. "Compared to individuals with non-elevated BMI levels, obese patients undergoing PCI had lower short- (OR 0.63; 95% CI 0.54–0.73) and long-term mortality (OR 0.65; 95% CI 0.51–0.83). Post-CABG, obese patients had lower short-term (OR 0.63; 95% CI 0.56–0.71) and similar long-term (OR 0.88; 95% CI 0.60–1.29) mortality risk compared to normal weight individuals" [17]. The authors concluded that the mortality rate after CABG did not differ significantly between the BMI groups. Finally, the results also suggest that a potentially protective relationship between obesity and mortality is absent in longer follow-up times or increasing severity of obesity.

In the era of risk stratification and informed consent, universally applied assessment of surgical risks may not adequately aim to evaluate obesity as a predictor in various scoring systems [23]. Based on the EuroSCORE model the BMI appears not to be considered as a potential risk factor [33, 34]. The STS scoring system includes low BSA as an independent risk factor (OR 0.91 per 1 unit). Florath et al. [28] attempted to modify the EuroSCORE algorithm by adding the risk factor 'BMI < 24' (with a coefficient established in their study) that lead to an increased c-index to 0.75 (0.67–0.82). They concluded that the prediction of the EuroSCORE algorithm could be improved by including the risk factor 'small BMI'.

The obesity paradox does appear to exist for high BMI patients with established CAD in whom prognosis appears to be generally equal-to or even superior to low or normal BMI patients [35, 36]. This paradox needs to be further evaluated and outcome integrated into modern risk scoring systems. It may be that the mortality after CABG is increased for patients with an extreme BMI in either the low or the high range [15]. A consensus of whether small BMI or high BMI is associated with an increased risk is required in order to be integrated into the outcome prediction model.

Several limitations of this study are currently noted. The study evaluated only the short-term outcome. A longer follow-up period may have detected additional events influencing the final results. The study offers no data on the number of other comorbid conditions and potentially confounding factors such are fitness, neurologic disease, cancer, HIV status, smoking history, pulmonary hypertension, heart failure status, pre-surgical weight loss history, alcohol/illicit drug abuse, cardioprotective medication use, markers of inflammation, and clotting factors. Adiposity was assessed through BMI including no waist circumference, nor waist-to-hip ratio.

Conclusion

Whether obesity directly affects the outcome and prognosis of CABG is still the subject to considerable debate. Although the authors found no relationship between obesity and early postoperative mortality, it remains advisable for patients to reduce their weight to normal range. Obesity is a well-known risk factor for developing numerous cardiovascular diseases. Obesity can also be considered a predictor of elevated risk of postoperative complications. Further research is required to study the impact of obesity on the intermediate and long-term outcomes in patients after CABG.

Acknowledgements

The authors would like to thank Mr. Vojin Miladinov for the translation of this manuscript.

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Received on March 3, 2012.
Revised on March 20, 2012.
Accepted on April 3, 2012.

ORIGINAL ARTICLE

UDC: 616-072.1::616.348/.351-08
DOI: 10.2298/VSP120424040G

Endoscopic mucosal resection of flat and sessile colorectal adenomas: Our experience with long-term follow-ups

Endoskopska mukozna resekcija ravnih i sesilnih kolorektalnih adenoma: naše iskustvo sa dugotrajnim praćenjem

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Abstract

Bacground/Aim. Endoscopic mucosal resection (EMR) or mucosectomy is a removing method of flat or sessile lesions, laterally spreading tumors and carcinoma of the colon or the rectum limited to mucosa or the surface part of the submucosa. The aim of the study was to estimate the efficacy and safety of EMR in removing flat and sessile colorectal adenomas. **Methods.** This prospective study involved 140 patients during the period of 8 years. A total of 187 colorectal adenomas were removed using the EMR method "inject and cut with snare". **Results.** The approximate size of mucosectomised adenomas was 13.6 mm (from 8 mm to 60 mm). There was a total of 48 (25.7%) flat adenomas and 139 (74.3%) sessile adenomas, ($p < 0.01$). Using "en bloc" and "piecemeal" resection, 173 (92.5%) and 14 (7.5%) of colorectal adenomas were removed, respectively. In all the cases, a complete removal of colorectal adenomas was achieved. Two (1.4%) patients had adenoma removal with intramucosal carcinoma each. In the average follow-up period of 21.2 ± 17.8 months, 2 (1.4%) patients had adenoma relapse after EMR. Considering complications, there was bleeding in 1 (0.7%) patient with a big rectum adenoma removed with EMR. Furthermore, one (0.7%) patient had a postcoagulation syndrome after cecal adenoma was removed by EMR. **Conclusion.** EMR is an efficient, safe and minimally invasive technique of removing flat and sessile adenomas in the colon and the rectum, with a very low percentage of adenoma recurrence over a long period of monitoring.

Key words:
adenoma; colorectal neoplasms; endoscopy, digestive system; treatment outcome.

Apstrakt

Uvod/Cilj. Endoskopska mukozna resekcija (EMR) ili mukozektomija je metoda uklanjanja ravnih i sesilnih lezija, tumora sa lateralnim širenjem i karcinoma kolona i rektuma ograničenih na mukozu i površni nivo submukoze. Cilj rada bio je da se izvrši procena efikasnosti i bezbednosti EMR u uklanjanju ravnih i sesilnih adenoma kolona i rektuma. **Metode.** Tokom perioda od 8 godina prospektivnom studijom obuhvaćeno je 140 bolesnika kod kojih je tehnikom „ubrizgaj i seci sa omčom" metode EMR uklonjeno 187 kolorektalnih adenoma. **Rezultati.** Prosečna veličina mukozektomiranih adenoma iznosila je 13,6 mm (od 8 mm do 60 mm). Ukupno je bilo 48 ravnih (25,7%) i 139 sesilnih adenoma (74,3%) ($p < 0,01$). „En bloc" resekcijom uklonjeno je 173 (92,5%) kolorektalnih adenoma, dok je resekcijom deo po deo uklonjeno 14 (7,5%) adenoma. Kod svih bolesnika postignuto je potpuno uklanjanje kolorektalnih adenoma. Kod dva (1.4%) bolesnika uklonjen je po jedan adenom sa intramukoznim karcinomom. U prosečnom periodu praćenja od 21,2 ± 17,8 meseci, kod dva bolesnika (1,4%) je nakon EMR došlo do recidiva adenoma. Od komplikacija, krvarenje nakon EMR bilo je kod jednog bolesnika sa velikim adenomom rektuma (0,7%). Takođe, kod jednog (0.7%) bolesnika, nakon EMR adenoma cekuma došlo je do nastanka postkoagulacionog sindroma. **Zaključak.** Mukozektomija je efikasna, bezbedna i minimalno invazivna metoda uklanjanja ravnih i sesilnih adenoma kolona i rektuma, sa vrlo niskim procentom recidiva adenoma tokom dugotrajnog perioda praćenja.

Ključne reči:
adenom; kolorektalne neoplazme; endoskopija, gastrointestinalna; lečenje, ishod.

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Introduction

Colorectal cancer is the most frequent form of digestive system cancer. In most cases, it is caused by malignant transformation of adenoma (adenoma-carcinoma sequence). The evolution of the normal mucosa through adenoma into cancer is estimated to take from 5 to 20 years. Endoscopic removal of adenomatous polyps can reduce 80% of the colorectal cancer incidence [1–3].

Since about 80–90% of adenomatous lesions are smaller than 10 mm and in polypoid shape, the conventional snare polypectomy has been used for over three decades as an acceptable endoscopic method of lesion removal. However, sessile and flat lesions (their Paris classification being type 0-Is and 0-II) require a special skill as well as the experience of the endoscopist to use endoscopic mucosal resection (EMR) or mucosectomy [3]. EMR was first described by Deyhle et al. [4] in 1973, as an endoscopic technique of sessile colonic polyp resection. This technique proved efficient in removing flat colonic lesions or sessile that are bigger than 10 mm, as well as the laterally-spreading tumors of granular type [5, 6]. Non-granular laterally-spreading tumors have a greater malignant potential and can be removed only "en bloc", using the endoscopic submucosal dissection (ESD), the most developed in Japan [7]. With colorectal cancer spreading over the mucosa and surface level of the submucosa (sm1) there is a very low risk for metastasis in lymphic nodes, which absolutely indicates to EMR or ESD [8, 9].

There are various suction and non-suction techniques of EMR. The non-suction technique "inject and cut with snare" is most frequently used in the colon, and it represents submucosal injection of different solutions to elevate the lesion, which is then snared and electroresected. The elevation of a lesion makes easier putting it into the snare and prevents deeper layers of the colon from getting damaged during the electroresection. Lesions smaller than 20 mm are removed in one go ("one piece" resection), while those bigger than 20 mm get removed step by step ("piecemeal" resection) [10].

The aim of this prospective nonrandomized study was to estimate the efficiency and the safety of EMR in removing flat and sessile adenomas of the colon and rectum, as well as assessment of adenoma recurrence over a long period of monitoring.

Methods

In General Hospital Lekovac, Department of Gastroenterology and Hepatology, in the period of 8 years (2004–2011), a total of 187 colorectal adenomas were removed by EMR in 140 patients (98 men and 42 women), whose average age was 62.4 ± 10.12 years (from 42 to 80 years).

Criteria excluding patients from the study were the presence of nonadenomatous histological structure of mucosectomised lesion, synchronous cancer and significant comorbidity of the patients.

All the patients were asked to give their consent to colonoscopy and endoscopic removal of adenomas. Colonoscopic examinations were carried out with video colonoscopes of the Pentax and Olympus brand. The EMR method "Inject and cut" technique was used for the removal of a sessile (according to the Paris clasiffication of type 0–Is) and flat neoplastic lesions (type 0–IIa and 0–IIb). The standard 23 gauge and 4 mm long needle was used for submucosal application of the dilute saline solution of epinephrine (1:100,000). In some cases, we used indigo carmine (0.04%), or a few drops of methylene blue (0.5%) in a dilute solution of epinephrine, which contributed to a better demarcation of the elevated lesion. The total volume of the dilute epinephrine amounted from 4 mL to 20 mL. Submucosal injection was first applied underneath the proximal foreign lesion, then underneath the distal side, until the submucosal "pillow" was formed and lesion elevated. Then we placed an oval or hexagonal polypectomy snare, which got the elevated lesion snared and electroresected by the help of the electrosurgical unit. Resection was performed by cutting combined with electrocoagulation of the lesion (Figures 1–4). The size of adenoma was estimated by the familiar estimate on the basis of the scope of the open biopsy scissors. When adenomas were up to 20 mm in size "en bloc" resection was performed, whereas with adenomas larger than 20 mm we performed "piecemeal" resection. In the case of „piecemeal" resection, before getting each lesion snared a new quantity of the dilute solution of epinephrine was applied submucosally, so as to elevate the lesion.

Fig. 1 – Lagre sessile adenoma of the colon.

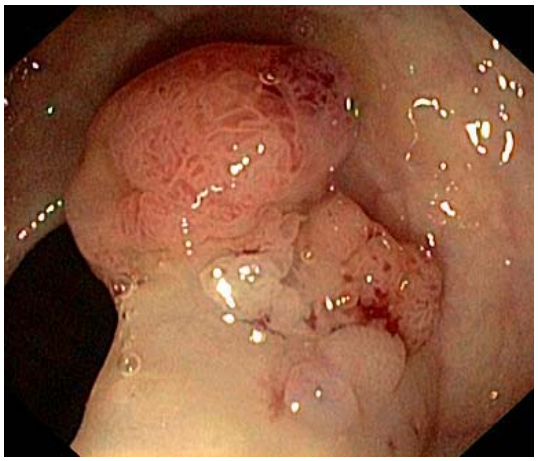

Fig. 2 – Adenoma elevation with submucosal injection of diluted epinephrine.

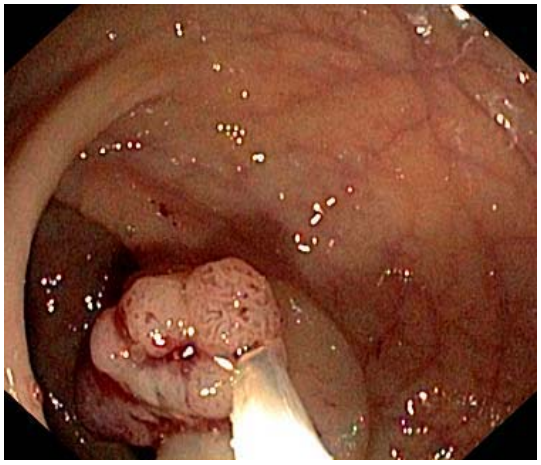

Fig. 3 – Adenoma electroresection with snare.

Fig. 4 – Lager ulceration after a complete adenoma resection.

Patients with synchronous polyps had the polyps removed with common polypectomy techniques, such as "cold forceps" polypectomy, "hot forceps" polypectomy, "cold snare" polypectomy and the common electrosection of polyps with a snare.

After EMR, endoscopic estimate was made to see if the adenoma was completely eliminated. The remaining adenoma tissue was removed by a new electroresection with a snare, and in case it was smaller than 5 mm, by "hot biopsy forceps". The material was sent to the Pathology Department, where the standard histology protocols of staining with hematoxylin and eosine were performed.

After having colorectal adenomas removed with endoscopy, patients subsided one or two days on a liquid diet, and the following day the intake of food was normal. After the endoscopic intervention, they were checked colonoscopically, for the residue or recedive estimate, once in three or six months, depending on the histologic findings about the removed adenoma. The next three years, colonoscopy examination was done once a year, and then at a longer interval of time. The average follow up was 21.2 ± 17.8 months (3 to 72 months).

For statistical analysis we used measures of central tendency (average value, $\bar{x}$) and measures of dispersion [standard deviation (SD)]. The method used to assess the significance of difference was Pearson's χ^2 test with Yates's correction. The existence of statistically significant differences between individual characteristics was considered at values of $p < 0.01$.

Results

Most frequently, there was one mucosectomy per a colonoscopic examination, in 103 (73.5%) of the patients, whereas the largest number of performed mucosectomies per patient, in a course of a single colonoscopy, was 5. Nine mucosectomies were performed on a patient in the course of 4 colonoscopic examinations. "En bloc" resection was used to remove 173 (92.5%) of colorectal adenomas, whereas "piecemeal" resection 14 (7.5%) were removed adenomas. In all the cases mentioned, colorectal adenomas were completely removed.

In 40/140 (28.5%) patients submitted to EMR using colonoscopy, 60 synchronous polyps were discovered, and endoscopycally removed.

The approximate size of the mucosectomised adenomas was 13.6 mm (from 8 mm to 60 mm). All mucosectomised sessile colonic adenomas were greater than 10 mm.

Regarding their endoscopic shape, there was a total of 48 (25.7%) flat and 139 (74.3%) sessile adenomas, which represents a statistically important difference ($p < 0.01$). Considering of their location, there was a statistically significant higher incidence of sessile adenomas in the rectum and the sigmoid part of the colon ($p < 0.01$), while in the other segments of the colon there was no statistically significant difference in endoscopic shape of adenomas ($p > 0.01$) (Table 1).

Table 1

Endoscopic shape of mucosectomised adenomas depending on their location

Endoscopic shape	Rectum n = 51	Sigma n = 77	Descendens n = 6	Transfersum n = 28	Ascendens n = 18	Caecum n = 7	Total n = 187
Flat, n (%)	11 (21.6)	16 (20.7)	1 (16.6)	11 (39.2)	7 (38.8)	2 (28.5)	48 (25.7)
Sessile, n (%)	40 (78.4)*	61 (79.2)*	5 (83.3)	17 (60.7)	11 (61.1)	5 (71.4)	139 (74.3)*

*$p < 0.01$ compared to other groups *vs* flat adenomas (χ^2 test)

Histologically, the largest number of removed adenomas were of tubular structure (128, 68.4%), then of tubulovillous structure (57, 30.5%), and the least frequent among them were villous (2, 1%), which was a statistically significant difference ($p < 0.01$). Regarding their location, it was statistically significant that tubular structure of adenoma occured more frequently in the rectum, the sigmoid section of the colon, the colon transversum and ascendens ($p < 0.01$), while only in the descendens and the caecum the difference

was not statistically significant in histologic structure of adenoma ($p > 0.01$) (Table 2).

Table 2

Histological structure of mucosectomised adenomas depending on their location

Histologic structure	Rectum n = 51	Sigma n = 77	Descendens n = 6	Transfersum n = 28	Ascendens n = 18	Caecum n = 7	Total n = 187
Tubular adenoma, n (%)	29 (56.8)	55 (71.4)	5 (83.3)	22 (78.5)	13 (72.2)	4 (57.1)	128 (68.4)
Tubulovillous adenoma, n (%)	21 (41.2)	21 (27.2)	1 (16.7)	6 (21.4)	5 (27.8)	3 (42.8)	57 (30.5)
Villous adenoma, n (%)	1 (1.9)	1 (1.3)	0	0	0	0	2 (1)

*$p < 0.01$ *vs* other groups (χ^2 test)

All the removed adenomas were mainly of the second-degree dysplasia (97, 51.9%), then third-degree (59, 31.5%), and, least frequently, first-degree (31, 16.6%), which was statistically significant, $\chi^2 = 34.16 > [\chi^2 (2 \text{ and } 0.01) = 9.21$, $p < 0.01]$. In 2 (1.4%) patients with adenoma removed each, severe dysplasia with intramucosal cancer focus was detected histologically.

In the average follow-up period of 21.2 ± 17.8 months in 2 (1.4%) patients after EMR occurred adenoma recurrence (Table 3). In 1 patient, after 18 months occured relapse of a big sessile adenoma on the hepatic flexure of the colon. Recurrent adenoma had tubulovillous structure with third-degree dysplasia, which was a finding identical to the one of the initial adenoma. Recurrent adenoma was then endoscopically removed, and twelve months after the removal there was no relapse. In the other patient with large sessile adenoma in the rectum, about 50 mm in diameter, 12 months after the "piecemeal resection", there was a recurrence which was completely removed endoscopically. Histologic findings of the removed relapse were identical to the ones of the original tumor – tubular adenoma with a third-degree dysplasia.

Table 3

Endoscopic mucosal resection outcomes

Outcome	Patients n (%)
Adenoma relapse	2 (1.4)
Complications	
bleeding	1 (1.7)
post coagulation syndrome	1 (0.7)

As far as complications are concerned (Table 3), there was bleeding after mucosectomy in 1 (0.7%) of the patients with a big sessile adenoma in the rectum, which had been removed with a "piecemeal" resection. The bleeding was stopped by placing 6 clips. After mucosectomy of a flat adenoma in the caecum, with one (0.7%) patient there occured the postcoagulation syndrome with abdominal pain and pericaecal infiltration. The patient was operated on, but later the existent liver and kidney insuficiency worsened, leading to lethal outcome.

Discussion

Today, EMR represents a rutinary procedure of removing flat, larger sessile lesions, as well as laterally spreading tumors in the colon and the rectum. EMR has an advantage over the traditional surgical methods for malignantly alterated colorectal adenoma and cancer, because of a lower rate of procedural morbidity and mortality [11]. This method can remove cancer spreading over mucosa and the surface level of the submucosa, deep to 1,000 μm (sm1 invasion level), on condition that the cancer has been well histologically differentiated, and there is no lymphatic or venous invasion. This is particularly important with elderly patients with significant comorbidity, where the risks of surgical interventions are high [7, 12, 13].

There are various suction and non-suction techniques of EMR. Suction techniques run a higher risk of affecting the muscular layer, especially in the colon and the rectum, so the submucosal application of a larger amount of different solutions and a controlled power of suction are recommended [14]. Safer for the colon and the rectum are the non-suction EMR techniques. The non-suction technique "inject, lift and cut", where lesion gets elevated by forceps before resection, requires a double-channel endoscope [15]. Still, "inject and cut with snare" is the most frequently used technique for the colon and the rectum, and we used it for our patients, too. The essence of this technique is to use an injection of different solutions submucosally, underneath the proximal and then the dystal part of the lesion, in order to elevate it, which enables its snaring and electroresection. Elevation of the lesion thickens the colon wall and the refore prevents the deeper layers of the wall from getting damaged during electroresection [16].

For submucosal injections of the EMR "inject and cut with snare" technique we used a 1 : 100,000 physiological saline solution with epinephrine, which was used most frequently. Epinephrine dilution of 1 : 200,000 is also used. Attenuated epinephrine has no role in preventing delayed bleeding but is useful for creating a bloodless field during EMR. Furthermore, epinephrine limits the submucosal solution dispersion field which contributes to a longer elevation [3]. Some studies have shown the advantage of the usage of other solutions for submucosal injection, such as hyaluronic acid, hydroxypropylmethylcellulose, glycerol and succinylated gelatin, which contribute to a longer elevation. The disadvantage of these solutions is their price and not being available to many endoscopic units [3, 17]. Besides, the use of hydroxypropylmethylcellulose, as well as other solutions like hypertonic sodium chloride (3.75%) and dextrose (20%) may harm the tissue and cause local inflammatory reactions [7].

In some cases, we applied indigo carmine or methylene blue in a diluted solution of epinephrine. The use of biologically inert color of indigo carmine and methylene blue provides good elevated lesion demarcation and well-defined

safety of the resection line. The color does not tie into the muscular layer, so in case this layer gets involved in resection, there can be observed a discontinuum in the uniform blue of submucosis. Furthermore, adenoma tissue, which is red, can clearly be identified. The use of color eliminates the need for thermal marking of the resection margins. A special benefit of the use of color is with lesions which are not clearly separated, such as laterally spreading tumor and sessile serrated adenoma [18].

We applied a total of 4–20 mL diluted solution of epinephrine submucosally. In "piecemeal" resection of adenomas larger than 20 mm, before each snaring we applied a new amount of epinephrine solution submucosally in order to elevate the lesion. According to literature, the total volumen of the applied solution during EMR amounted to 5–50 mL. "Non-lifting sign" signaled that structures deeper in the colon wall were affected by tumor and such patients were sent to laporascopic or open surgical resection [3].

According to the literature, complete removal of colorectal adenomas is achieved in 75–100% cases [6, 8, 19–22]. Sucess of the EMR technique depends on adenoma size and depth of the invasion. Laterally spreading tumors larger than 30 mm are rare and require a special skill for a safe removal. They can hardly be removed "en bloc", usually they are removed "piecemeal". According to a study[19], "en bloc" resection was achieved in 73.1% of tumors smaller than 30 mm, and only 15.4% of tumors larger than 30 mm. A high success (100%) of complete adenoma removal in our patients can be explained by the fact that intramucosal cancer in adenoma was found with only two (1.4%) patients and the fact that the average size of the removed adenoma (13.6 mm) was smaller in comparison to other series [6, 8, 19–22].

Removed adenomas in our patients were significantly more sessile (74.3%) than flat adenomas (25.7%). Regarding their location, sessile structure of adenomas was significantly more likely to be present in the rectum and the sigmoid part of the colon, while in other segments, despite a high occurence of sessile adenomas in comparison to flat adenomas, the difference was not statistically significant. According to the literature, flat adenomas are found in 6.8% to 36% of all adenomas [23–25].

The largest number of adenoma are of tubular structure (80–86%), then tubulovillous (8–16%), and least frequently villous (3–16%) [26]. A similar ratio was there with our series of examinees. According to the literature, adenomas most frequently show mild dysplasia (70–86%), then moderate dysplasia (18–20%), and the least frequent severe dysplasia with carcinoma *in situ* (5–10%) or invasive carcinoma (5–7%) [27]. In our examinees, adenomas most frequently had moderate dysplasia, then severe dysplasia and the least frequent mild dysplasia.

After extensive piecemeal EMR, it is advised that colonoscopic examinations take place from 3 to 6 months, then once a year over the next 3 years, and then at longer intervals [3]. Our patients underwent similar examinations. Adenoma relapse after EMR ranges from 0–46%, depending on adenoma size and follow-up period. A higher risk for relapse was observed in adenomas larger than 35 mm. In case of visible adenoma residue after EMR, use of argon plasma coagulation reduces the frequence of relapsing adenoma for 50% [28]. In order to provide a long term monitoring of our patients after mucosectomy, we excluded from the study the patients with a significant comorbidity and synchronous cancer. In both (1.4%) our patients with relapse, adenoma was too of a larger size. We removed residual adenoma tissue by electroresection with a snare or "hot biopsy forceps".

Perforation after EMR was shown in 1–2% of cases and bleeding in about 2% of the cases. These complications can be successfully endoscopically managed with hemoclips [21, 29]. We had 1 (0.7%) examinee with bleeding following mucosectomy of a large rectal adenoma, which was sanated with hemoclips. Post-coagulation syndrome occurs because of transmural bowel wall damage in 0.51–3.7% of cases. It manifests most frequently from the first to the fifth day after the polypectomy, with the symptoms of localised abdominal pain, febrility, peritonitis and leukocytosis. Conservative therapy is usually sufficient for resolution of the symptoms [22, 30]. In one (0.7%) of our patients, after caecal adenoma mucosectomy appeared post coagulation syndrome, which could not be sanated with drug treatment, and the patient was operated on.

Conclusion

With well-defined indications, EMR is an efficient, safe, as well as minimally invasive method of removing flat and sessile adenomas in the colon and rectum, and in many cases it has replaced surgical methods. During a long follow-up period after complete removal of adenoma there is a very low percentage of their recurrence, which is important in secondary prevention of colorectal carcinoma.

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Received on April 24, 2012.
Accepted on August 23, 2012.
OnLine-First August, 2013.

ORIGINAL ARTICLE

UDC: 617.584/.585-089.844
DOI: 10.2298/VSP1401039T

Clinical aspects of reconstruction of the lower third of the leg with fasciocutaneous flap based on peroneal artery perforators

Klinički aspekti rekonstrukcije distalne trećine potkolenice fasciokutanim režnjem baziranim na perforatorima peronealne arterije

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Abstract

Background/Aim. Fasciocutaneous flaps are tissue flaps that include the skin, subcutaneous tissue and underlying fascia, and are based on the septocutaneous system of blood vessels. They have a number of characteristics that give them an advantage in many cases, especially in treatment of lower extremity defects. The aim of this study was to clinically analyse the outcomes of patients surgically treated with *a. peroneae* perforator-based reverse flaps of the lower leg. **Methods.** This prospective study was conducted on a group of 22 patients with tissue defects in the lower third of the lower leg and foot. All the 22 patients underwent standard diagnostic procedures and the preoperative preparation for spinal anesthesia. During the surgery, the location of the recipient site with a future flap and an accompanying vascular stem was marked. Having prepared the recipient site in accordance with the surgical principles, the lobe was prepared, rotated and positioned in the defect region. **Results.** The proportion of male to female patients in our study group was 19 (86%) to 3 (14%), respectively. The maximal defect frequency (14 or 63%) was found in the age group of 30–50 years, i.e. working-age people. Trauma in 16 (72%) of patients was the most common reason for surgical treatment. The most common defects our patients had were found in the distal third of the leg (5, 22%), medial malleolus (4, 18%), the dorsum of the foot (3, 13%) and the foot (4, 18%). The number of lobes (18, 81%) applied in the course of our clinical study was statistically more significant ($p < 0.05$) than the number of lobes applied along with the skin autografts (4, 19%). During the final follow-up of the applied lobes, 6 (27.2%) of early and 2 (9%) of late complications were observed, making a total of 36.2% of complications occurring in the applied lobes, of which only 1 was lost. **Conclusion.** Clinical application of *a.suralis superficialis mediani*-based reverse flap is justified by a high percentage (95.5%) of preserved lobes. Functional recovery of Achilles tendon injuries treated with the fasciocutaneous flap appeared to be effective. The results obtained during the clinical investigation can be very helpful in using this flap more optimally and more often in a wide range of different reconstructive surgical procedures in the field of plastic surgery.

Key words:
reconstructive surgical procedures; lower extremity; surgical flaps; recovery of function.

Apstrakt

Uvod/Cilj. Fasciokuatni režnjevi su režnjevi koji u svom sastavu imaju kožu, potkožni sloj i fasciju, a bazirani su na septokutanom sistemu krvnih sudova. Imaju niz karakteristika koje im daju prednost kod velikog broja povređenih, a posebno u zbrinjavanju defekata na ekstremitetima. Cilj ove studije bila je klinička analiza rezultata operativnog hirurškog lečenja bolesnika kod kojih su korišćeni reverzni režnjevi potkolenice bazirani na perforatorima *a. peroneae*. **Metode.** Ova prospektivna studija sprovedena je na 22 bolesnika sa defektom tkiva na zadnjoj strani donje trećine potkolenice i stopala kod kojih su urađene preoperativno standardne dijagnostičke analize i priprema bolesnika za spinalnu anesteziju. U operativnom postupku markirano je mesto davajuće regije sa budućim režnjem i pripadajućom vaskularnom peteljkom. Režanj je ispreparisan, rotiran i postavljen na mesto defekta uz predhodnu pripremu primajuće regije shodno hiruškim principima. **Rezultati.** U ovom kliničkom ispitivanju dominirali su bolesnici muškog pola, 19 (86%), dok je žena bilo 3 (14%). Najveća učestalost defekata, 14 (63%), bila je u starosnoj grupi od 30–50 godina, tj. kod radno sposobnih ljudi. Trauma, 16 (72%), bila je najčešći razlog za operativno liječenje. Defekt se najčešće nalazio na distalnoj trećini potkolenice, 5 (22%), medijalnom maleolusu, 4 (18%), dorzumu stopala 3 (13%) i peti 4 (18%).

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Broj primijenjenih režnjeva, 18 (81%), u toku kliničkog rada bio je statistički značajniji ($p < 0,05$) nego broj primijenjenih režnjava zajedno sa autotransplantatom kože, 4 (19%). U toku praćenja primenjenih režnjeva bilo je 6 (27,2%) ranih komplikacija i 2 (9%) kasne što iznosi ukupno 36,2% komplikacija na primenjenim režnjevima, od kojih je samo jedan bio izgubljen. **Zaključak.** Primena reverzibilnog režnja baziranog na *a. suralis superficialis medianae* je opravdana zbog velikog procenta (95,5%) očuvanih režnjeva. Funkcionalni oporavak kod povreda u predelu Ahilove tetive pri primeni fasciokutanog režnja bio je efikasan. Dobijeni rezultati mogu biti višestruko korisni u plastičnoj hirurgiji radi optimalnije i češće upotrebe ovoga režnja u različitim rekonstruktivnim hirurškim zahvatima.

Ključne reči:
hirurgija, rekonstruktivna, procedure; potkolenica; režnjevi, hirurški; funkcija, povratak.

Introduction

Fasciocutaneous flaps are tissue flaps that include the skin, subcutaneous tissue and underlying fascia, and are based on the septocutaneous system of blood vessels. They have a number of characteristics that give them advantages over other types of flaps, especially in the management of the lower extremity defects. A fasciocutaneous flap is vascularized by fasciocutaneous (septocutaneous) blood vessels originating from the regional blood vessels, that pass through an intermuscular and intercompartmental fascial septa to the surface of the fascia, where they branch off to form the plexuses which deliver the blood to the subcutaneous fatty tissue and the skin of the region above the perforator. The most important feature of a fasciocutaneous flap is the vascularization system which distinguishes it from the other flaps [1, 2]. The distally-based superficial sural artery flap was been first described in the experimental study of Masquelet et al [3]. It is a skin island flap supplied by the vascular axis of the sural nerve. Arterial blood supply to the flap is provided by the superfacial sural artery which anastomoses with septocutaneous perforators *a. peroneae*. The pivot point of the pedicle must be at least 5 cm proximal to the lateral malleolus [4, 5]. Hasegawa et al. [6] presented the successful use of that flap in 21 patients on the basis of the results published in the 90s of the last century [3]. During the postoperative period, the largest number of patients had venous stasis flaps, of which the largest ones (10 × 13 cm) were maintained for two months. A more favorable clinical course was observed in patients with smaller flaps. There were two great advantages of that flap. The first referred to the easy and fast surgical preparation and flap design without doppler diagnosis, while the second referred to the fact that there was no need to sacrifice the main arteries of the lower leg [7, 8]. The flap size, location, width as well as esthetic requirements of the recipient rergion should be taken into consideration during planning and designing [8, 9]. The flap was then outlined and the vascular pedicle marked 5 cm proximally to the lateral malleolus, where the point called the "pivot point" of the flap was marked. Further, the flap was designed along the medial line of the lower leg which represented the path of the neurovascular pedicle.

The aim of the study was to analyze clinical outcomes of surgical treatment with reverse lower leg flaps based on *a. peroneae* perforators.

Methods

This prospective study on a group of 22 patients with tissue defects in the lower third of the lower leg and foot was conducted in the Military Medical Academy, Belgrade, Serbia the Clinic for Plastic Surgery, the Clinical Center of Montenegro, Podgorica, Montenegro and the Surgical Ward, Foča General Hospital, Foča, Bosnia and Herzegovina.

All the 22 patients with the defect in the lower third of the lower leg and proximal part of the foot underwent standard diagnostic procedures and the preoperative preparation for spinal anesthesia.

During the surgery itself, we marked the location of the recipient site with a future flap and accompanying vascular stem.

After the recipient area has been prepared, accordind to surgical principles of this procedure, the lobe was prepared, rotated and positioned in the defect region.

The functional and esthetic outcomes of the reconstruction method applied in all the 22 patients who underwent the lower leg reconstruction with reversible fasciocutaneous flap based on *a. suralis superficialis mediani* were postoperatively analyzed. Functional investigations referred to the evaluation of the mobility of the distal third of the lower leg and hinge joint, where the planned flap could ensure, through further rehabilitation, the functional recovery at the highest level possible.

All the obtained data were statistically processed upon their collection. The $p < 0.05$ was considered to be of statistical significance.

Results

During our clinical investigation period, a total of 22 patients of different ages and gender underwent surgery, i.e. reconstruction of the lower leg with the flap based on *a. suralis superficials mediani*.

During this clinical investigation there were more male than female patients in the study group, the ratio being 19 (86%) to 3 (14%), respectively.

The maximal sickness frequency (14 patients, 63%) was found in the age group of 30–50 years, i.e. working-age people, $p < 0.05$ (Figure 1).

Trauma was found to be the most common reason for surgical treatment in the course of this clinical investigation (16 patients, 72%), $p < 0.05$ (Table 1, Figure 2).

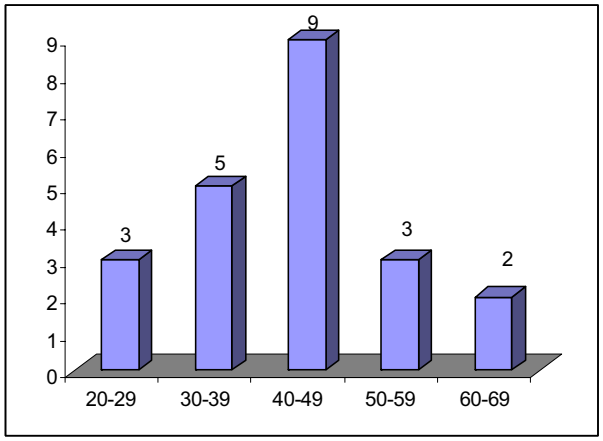

Fig. 1 – Age structure of the patients.

The most common defects were observed in the distal third of the leg (5 patients, 22%), medial malleolus (4 patients, 18%), the dorsum of the foot (3 patients, 13%) and the foot (4 patients, 18%) (Table 1).

A total of 18 (81%) lobes applied in the course of this clinical study was statistically more significant ($p < 0.05$) than the number of lobes applied along with the skin autografts, 4 (19%).

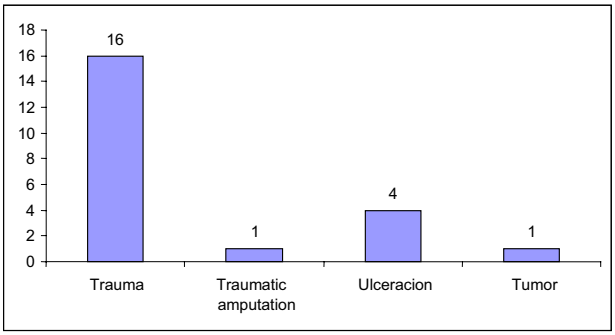

Fig. 2 – The types of injuries.

During the postoperative flap monitoring period, we observed 6 (27.2%) early and 2 (9%) late complications, making a total of 36.2% complications in relation to the applied flaps, of which only one was lost (Table 2).

Table 1

The size of fasciocutaneous arterial flap and defect dimensions and the application and transfer of flap through the subcutaneous tunnel or not through it

Patient's number	Cause of the defect	Size of the defect (cm)	Size of the lobe (cm)	Localization of the defect	Path
1	Trauma	9 × 6	11 × 8	Distal third of the leg	Not through the tunel
2	Trauma	10 × 6	12 × 8	Hell, *malleolus*	Not through the tunel
3	Ulceration	5 × 4	6 × 5	Med. *malleolus*	Through the tunel
4	Ulceration	5 × 4	6 × 5	Med. *malleolus*	Through the tunel
5	Trauma	5 × 5	7 × 6	Med *malleolus*	Not through the tunel
6	Trauma	9 × 8	11 × 7	Hell	Through the tunel
7	Trauma	7 × 6	9 × 7	*Malleolus*	Not through the tunel
8	Trauma	9 ×8	12 × 8	Distal third of the leg	Not through the tunel
9	Tumor	6 × 5	7 × 6	Hell	Through the tunel
10	Trauma	8 × 8	9 × 8	Distal third of leg	Not through the tunel
11	Ulceration	7 × 6	8 × 8	Med. *malleolus*	Through the tunel
12	Trauma	7 × 4	8 × 5	Distal third of the leg	Not through the tunel
13	Ulceration	5 × 4	6 × 4	Med. *maleolus*	Passage through the tunel
14	Trauma	10 × 6	11 × 7	Distal third of leg	Not through the tunel
15	Trauma	4 × 3	5 × 4	Hell	Through the tunel
16	Trauma	5 × 3	6 × 4	The defect in a part of the Achilles' tendon	Not through the tunel
17	Trauma	9 × 7	10 × 9	Dorsum of the foot	Not through the tunel
18	Traumatic amputation	12 × 8	15 × 10	Dorsal side of the foot with amputating stump of it	Not through the tunel
19	Trauma	7 × 6	10 × 7	Front side, distal third of the leg	Not through the tunel
20	Trauma	5 × 5	7 × 6	Hell	Through the tunel
21	Trauma	10 × 7	12 × 8	Dorsum of the foot	Not through the tunel
22	Trauma	9 × 7	10 × 8	Lateral *malleolus* and the hell	Through the tunel

Table 2

The presentation of immediate and late complications

Early complications	Patients n (%)	Late complications	Patients n (%)
Hematoma	2 (9)	Hypertrophic scar	1 (4.5)
Infection	1 (4.5)	Hyperkeratosis	1 (4.5)
Superficial flap necrosis	1 (4.5)		
Marginal necrosis	1 (4.5)		
Lost lobe (infection and necrosis)	1 (4.5)		

All the patients underwent a complete physical therapy. Functional recovery from Achilles' tendon injuries managed with the fasciocutaneous flap that was observed in our patients was much better.

Results of flap incorporation to the recipient defect site are presented in Figure 3.

Discussion

The methods of clinical investigation were classified into 3 groups: preoperative preparation, surgical technique

Fig. 3 – The flap postoperative follow-up to its final incorporation into the recipient defect site.
a, c, e – Recipient defect site preoperatively; b, d, f – Flap final incorporation into the recipient defect site.

for the fasciocutaneous flap, postoperative monitoring of the flap until its definite placement into the recipient defect site. Through surgical treatment, we showed the possibilities of and justification for the use of this flap in reconstruction of distal lower leg and foot defects. This surgical procedure was performed in the patients with the following indications: posttraumatic defects in the areas of the distal third of the lower leg on its medial and posterior side, the Achilles' tendon and the foot, the medial and lateral malleolus, and the dorsal side of the foot including the posttraumatic distal foot amputation; chronic ulcus in the area of calcaneus, and postoperative defects after tumor excisions.

The surgical procedure was performed after the standard preoperative preparations, biochemical tests, necessary cardiological examinations, color doppler imaging of the lower-extremity blood vessels and other required exams.

The surgical procedure was performed under spinal anesthesia with the patient in the pronate position, and intraoperative changing of the patient's position in relation to the defect localization.

The surgery started with devitalized injury tissue and skin edges debridement, and granulation tissue removal in cases of posttraumatic defects, or with vulnerable scarf, i.e. tumor excision, and the definite soft-tissue defect formation. After that, a footprint of the formed soft-tissue defect was taken over the sterile rubber material, and, after precise planning of the pedicle, used for covering the recipient site on the posterior side of the proximal lower leg, and for raising the planned flap slightly larger than the primary defect. Finally, the flap was elevated by using our own technique along with careful hemostasis and bipolar coagulation.

Particular care and attention was paid to the width of the neurovascular pedicle containing 1 cm deep fascia taken on either side of the pedicle to allow for better vasculariozation of the flap.

The flap was transferred to the prepared soft-tissue defect site through a subcutaneous tunnel or without passing it through the tunnel, taking care not to twist the pedicle. The edges of the flap were sutured together in one layer. The base of the flap, dependent on its width, was closed by direct undermining the skin flaps on the sides or using autotransplants. Direct closure of donor defect sites up to 6 cm in diameter was possible with the flaps equal in width, but if the skin island flap was wider, the donor site closure was possible with the Thiersch skin autotransplant. The skin graft was harvested from the opposite leg with the electric dermatome, placed in the donor site, and fixed with separate unresorbable sutures. It was covered with vaseline gauze, wet gauze and dry gauze in 4 layers. The graft was also dressed with vaseline gauze and dry gauze in 3 layers, and a circular hole cut out in the middle of it to monitor the graft vitality. After the surgery, a patient could lie on the left and the right side, but was not allowed to lean on the dorsal side of the operated lower leg.

During this investigation, there were more male than female patients, the ratio being 19 (86%) to 3 (14%), respectively. The maximal sickness frequency (n = 14, 63%) was found in the age group of 30–50 years, i.e. working-age people.

In the course of this clinical investigation, trauma 16 (72%) appeared to be the most common reason for the surgical treatment. The most frequent defects were found in the distal third of the leg (n = 5, 22 %), medial malleolus (n = 4, 18%), the dorsum of the foot (n = 3, 13%) and the foot (n = 4, 18%).

The flaps and defects we investigated were of various dimensions, ranging from 5 × 3 cm (injuries) to 12 × 8 cm (traumatic amputations). During surgical application of the flap, the length of the vascular pedicle ranged from 14 cm maximally to 9 cm minimally, with the average values of 11.5 cm and standard deviation of 6.5 cm. The flap mobility was extraordinary, with the possibility to rotate up to 180 degrees. Having in mind that the flap could be rotated in any direction, it was possible to cover any point of the full circle within the diameter of the maximum flap length, taking care not to twist the pedicle. The number of patients surgically managed using the flap passed via the subcutaneous tunnel was 9 (41%), while the number of those with the flap not passed through the tunnel to the site of application was 13 (59%).

The number of flaps used in the patients was 18 (81%), and the number of flaps applied together with the skin autografts was 4 (19%).

The donor sites in 9 (41%) of patients were closed directly, while the closure of the donor site in 13 (59%) of patients was done using autotransplants ($p < 0.05$).

Bleeding edges of the flap during the surgical procedure were the first sign of its vitality. During the immediate postoperative period, the vitality of the flap was monitored using the "refillig" test (a change in the flap color to redness upon releasing finger pressure or closed forceps) and bleeding upon the insertion of the insulin needle. Temporary venous congestion of the flap was seen in distally based flaps [10, 11]. It occurred due to the retrograde flow of venous blood, and the retrograde position of the valves in the venous blood vessels [12, 13]. Venous drainage of the transferred flap was monitored by evaluating the two parameters, that is, the flap color and capilary refilling. The color of the flap was assessed by comparing it with the color of the adjacent skin. If the color was the same as that of the adjacent skin, it was regarded as normal (blanchable red). If it was darker when compared with the color of the adjacent skin, it was assessed as livid. The lividity of the flap, and the adenoma developed in the later stage were caused by venous congestion. Adenoma sometimes exists for up to two months. Similar results were reported by the other authors [14–17]. The reason for venous congestion of the distally based flap lies in the fact that venous drainage is carried out through small veins running parallel with the small saphenous vein.

Venous congestion is considered to be an accompanying postoperative phenomenon by many authors [18–20]. On one side, they bypass the adjacent valves of the large superficial veins, and on the other side, they anastamose with the perforator concomitant veins through which the definite drainage is carried out. The function of the fascious plexus in the venous drainage is emphasised here [19, 20]. The flap refill test

performed by applying thumb pressure was assessed normal when the color returned within 3 seconds, indicating, thus, that the capillary blood flow in the flap was normal. If color change took more than 3 seconds, capillary refill was considered to be slow.

In the postoperative period, the status of the recipient site was inspected regularly through dressing changes. However, care was given to the donor sites of both the flap and free skin graft. Aspiration drainage of directly closed donor sites was performed after 48 hours, while running sutures used to close the wound were removed on the day 14 postoperatively. Some authors report earlier removal of sutures, and later removal of a drainage tube depending on its activity [19, 20]. Following complete epitalization, the donor sites closed with the free Thiersch's skin grafts were controlled, and the dressings removed from the donor sites between the day 15 and the 20 postoperatively.

The grafts applied on the donor sites were dressed on the day 2 after the surgery to ensure they were properly attached to the site, that no blood accumulated beneath the grafts and that there was so partial nor complete graft lisis. The adherence to the donor site was achieved within 10 days, when the sutures used to hold the graft in place were also removed. Dressing changes were performed not later than 28 days to ensure complete healing of both the donor site and the autotransplant. In the patients with bone fractures, callus formation at fracture site and fracture healing were submitted to radiography and regular orthopedic controls. Callus formation at sites of fractures has been described by other authors as well [20–22]. On the basis of the radiography and clinical findings, the inserted osteosynthetic material was removed. Depending on the histopathological findings, regular postoperatove follow-ups were indicated to prevent reccurence of recedives.

During the postoperative monitoring of the flap, 6 (27.2%) early and 2 (9%) late complications were observed, making together a total of 36.2% complications in relation to the applied flaps, of which only 1 was lost. The postoperative follow-up of the applied flaps was also described by other authors who reported the total number of complications similar to determined in this investigation [21, 22]. The number of flap failures reported by other authors was larger due to the various numbers of applied flaps [23, 24].

The foot bones knited together can move to the left and to the right, and these movements play a very crucial role in the foot stability and balance. As a whole, the foot and its connection with the lower leg bones, in combination with some knee actions are considered a functional entity. Therefore, after a short resting period, the complete physiotherapy including electrotherapy, ultrasound therapy, magnetotherapy with the emphasis on the lymphatic drainage, massage and mobility exercises was initiated to reduce swelling as fast as possible. Depending on the extent of surgical procedure and concrete possibilities, the aim was to reestablish the normal range of the joint motion, and load it with the full-body weight.

All the patients underwent a complete physical therapy. The functional recovery from Achilles' tendon injury managed with the fasciocutaneous flap was shown to be considerably better than. Namely, long after all the symptoms removal, foot instability to a smaller or larger extend was demonstrated. It is the reason for indicating the functional treatment as to regain the lost abilities. It mostly includes strength, extension and balance exercises. Once the functional therapy has been completed, we could say that everything possible has been done to prevent new damage to the joint itself, to its functional components or surrounding physical structures.

Conclusion

The use of the reverse flap based on *a. suralis superficialis mediani* is justified by a high percentage (95.5%) of preserved lobes.

Functional recovery of Achilles tendon injuries treated with the fasciocutaneous flap was shown to be effective.

The results obtained during the investigation can be very helpful for using this flap more optimally and more often in a variety of reconstructive surgical procedures in the field of plastic surgery.

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Received on April 28, 2012.
Revised on July 2, 2012.
Accepted on July 3, 2012.

ORIGINAL ARTICLE

UDC: 616.31-085.46:616.311.2-0918.8-092.9
DOI: 10.2298/VSP1401046K

The rabbit gingival tissue response to retraction liquids and tetrahydrozoline

Odgovor gingivalnog tkiva kunića na retrakciona sredstva i tetrahidrozolin

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Abstract

Backround/Aim. Retraction agents for temporary vertical and lateral suppression of gingival tissue as well as bleeding control and fluid flow in the gingival sulcus are expected to have maximal efficiency without irreversible damage of local tissue and adverse systemic effects. The research started from the assumption that tetrahydrozoline is a biologically more acceptable means of gingival retraction than commercially available preparations. The aim of the study was to comparatively analyse the inflammatory effects of different retraction materials and tetrahydrozoline. **Methods.** The effect of retraction liquid on the basis of aluminum chloride and epinephrine and tetrahydrozoline hydrochloride on gingival tissue of rabbits was investigated. The application time in the rabbit's gingival sulcus was 7 minutes. Tissue biopsy was performed after an hour, a day, and 7 and 30 days. Tissue preparations were analyzed under a microscope. **Results.** The obtained results indicate a reversible damage of gingival tissues as a result of local application of aluminum chloride- and epinephrine-based retraction agents. Their use led to acute inflammatory response after an observation period of 1 and 7 days. After 30 days reparation of damaged tissue was observed. The use of tetrahydrozoline resulted in a visibly weaker inflammatory response. **Conclusion.** Retraction liquids insertion led to an acute inflammatory response of gingival tissue which in time assumed a chronic character. The inflammatory response to the administered tetrahydrozoline was significantly lower with complete reparation of gingival tissue. Taking this fact into account it is recommended as a potential retraction agent.

Key words:
gingivitis; tissues; inflammation; animals, laboratory.

Apstrakt

Uvod/Cilj. Retrakciona sredstva za privremeno vertikalno i lateralno povlačenje gingivalnog tkiva i kontrolu krvarenja i protoka tečnosti u gingivalnom sulkusu trebalo bi da imaju maksimalnu efikasnost bez ireverzibilnog oštećenja lokalnog tkiva i neželjenih sistemskih efekata. U istraživanju se krenulo od pretpostavke da je tetrahidrozolin biološki prihvatljivije sredstvo za gingivalnu retrakciju od komercijalno dostupnih preparata. Cilj rada bio je komparativna analiza inflamatornog odgovora na dejstvo retrakcionih materijala i tetrahidrozolina. **Metode.** Istraživan je efekat retrakcionih sredstava na bazi aluminijum-hlorida i epinefrina, kao i tetrahidrozolin-hidrohlorida na gingivalno tkivo kunića. Vreme primene ispitivanog materijala u gingivalni sulkus kunića iznosilo je 7 min. Nakon opservacionog perioda od 1 h, jednog, sedam i 30 dana vršena je biopsija gingivalnog tkiva i dobijeni preparati su mikroskopski analizirani. **Rezultati.** Utvrđeno je reverzibilno oštećenje tkiva gingive izazvanog lokalnom aplikacijom retrakcionih sredstava na bazi aluminijum hlorida i epinefrina. Njihova upotreba, nakon 1-dnevnog i 7-dnevnog opservacionog perioda, dovela je do akutnog inflamatornog odgovora. Nakon 30 dana uočena je reparacija oštećenog tkiva. Primena tetrahidrozolina imala je za rezultat značajno slabiji inflamatorni odgovor. **Zaključak.** Upotreba retrakcionih rastvora dovela je do akutnog inflamatornog odgovora tkiva gingive, koji je vremenom poprimio hronični karakter. Inflamatorni odgovor sa primenom tetrahidrozolina bio je značajno slabiji sa potpunim oporavkom gingivalnog tkiva. S obzirom na to tetrahidrozolin se može preporučiti kao potencijalni retrakcioni agens.

Ključne reči:
gingivitis; tkiva; zapaljenje; životinje, laboratorijske.

Introduction

Regular impression taking is a prerequisite for construction of high-quality fixed prosthetic appliance, thus allowing maximum accuracy possible at the contact site of biological tissue and restoration margin and ensuring integrity of periodontal structures. If a preparation margin is set at the level of or below the gingival margin, it is necessary to make it accessible to impression material by reversible temporary shift in apical direction.

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One of the most commonly used clinical methods for gingival retraction is a mechanical-chemical method which involves the use of special cotton cords of different thickness, impregnated with a solution (retraction agent) [1]. The role of retraction agent implies temporary vertical and lateral suppression of gingival tissue as well as bleeding control and fluid flow in gingival sulcus [2, 3]. For this purpose, vasoconstrictors (epinephrine) and astringents (aluminum chloride, aluminum sulfate, zinc chloride) are currently used. The applied retraction agent is expected to have maximal efficiency without irreversible damage of local tissue and adverse systemic effects [4].

On the other hand, some literature findings suggest that these gingival retraction agents may cause systemic reactions and local damage to periodontal tissues [5–7]. Systemic effect is related to epinephrine, especially if it is applied to damaged marginal gingiva and greater number of teeth, because it is contraindicated in patients with cardiovascular diseases, hyperthyroidism and diabetes [8, 9]. Since astringents act by precipitation of proteins and show very low cellular permeability they cause no systemic effects. Astringents of moderate concentrations cause irritation of surrounding tissue, and those of high concentrations cause caustic effect, which is especially important if one takes into account the fact that there is inadequate dose control [4].

Sympathomimetic vasoconstrictors also show retraction activity and are commercially available as nasal and olfactory decongestives [7]. Thus, these preparations having tetrahydrozoline as active component are also advantageous in dental prosthetics. In this study tetrahydrozoline was assumed to represent biologically more acceptable retraction agent when compared to commercially available products.

The aim of the study was to compare the effects of different commercially available retraction agents and tetrahydrozoline-based preparation on gingival tissue of rabbits.

Methods

The study included 3 commercialy available retraction agents and tetrahydrozoline-based agent (Table 1).

Experimental studies were carried out in accordance with the Helsinki Declaration (Approval of the Ethics Committee of the Faculty of Medicine in Niš, No. 01-2066-2).

The experiment involved 32 experimental male rabbits, 8 weeks of age and 1.8–2.2 kg of weight. The animals were divided into 4 experimental groups, 8 rabbits for each tested material. Each animal was administered 10 mg/kg intramuscular anesthesia Zoletil® (Virbac).

Experimental design was based on the following parameters: investigations were performed on gingival tissue of all 4 incisors of rabbits from each group; gingiva of the right incisors in all rabbits were the controls; gingiva of the left incisors were used for application of tested materials. Application was performed in gingival sulcus for 7 min using a retraction cord (Retracto®, Roeko) to enable even distribution of retraction agents. The impregnated cord was carefully placed along the whole tooth surface using plastic instrument and dental forceps. Gingival tissue of the upper right incisors in all the rabbits served as the negative control, *ie* it was intact tissue in the experiment. Gingival tissue of the lower right incisor in all the rabbits served as the control of the false treatment type, representing application of the retraction cord without tested material in the same way as it was used in the application of material. Simultaneous gingival biopsy of the left and right incisors of the jaw was done to avoid the influence of gingival biopsy injury of adjacent incisor.

The application plan, as well as observation period duration for each of the investigated retraction agents is shown in Table 2.

After 1 h, 1, 7 and 30 days of the treatment, gingival tissue samples were taken for histopathological analysis. Using a scalpel, 2 vertical incisions were made on the labial gingiva and one horizontal incision at the level of the alveolar ridge. Tissue samples of 2 × 2 mm were carefully separated in vertical direction by a raspatory. Upon biopsy tissue samples were fixed in 10% formaline. The material was further dehydrated in increasing concentrations of ethanol (from 50% to absolute). The material was illuminated by xylene and then put in paraffin molds. Tissue blocks embedded in periplast were cut on microtome (LKB Bromma, Sweden) (1.5 μL) and stained by hematoxylin & eosin (HE) method. Stained preparations were analyzed histopathologically on a NU-2 microscope (Carl Zeiss, Germany). The presence of collagen fibers was analyzed under polarizing light. Intensity of inflammation reaction was estimated semiquantitatively.

Table 1

Tested materials

Agent	Chemical content	Manufacturer
Retrargin®	25% aluminium chloride hexahydrate, pH = 0.8	Galenika, Serbia
Gingiva Liquid®	10% aluminium chloride hexahydrate, pH = 1.8	Roeko, Italy
Surgident® retraction solution	8% epinephrine -HCl, pH=2.5	Sigma Dental Systems Emasdi GmblH, Germany
Visine® Original	0.05% tetrahydrozoline hydrochloride, pH = 5.6	Pfizer, USA

Results

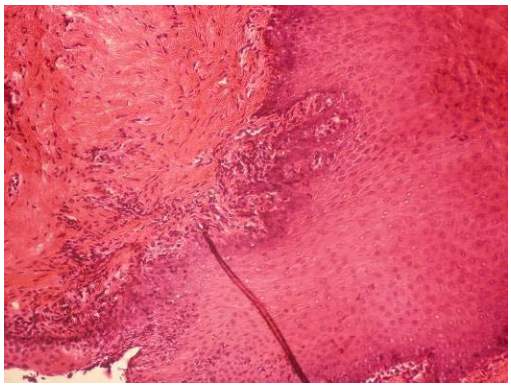
The tissue samples of clinically healthy gingiva of the negative controls that underwent no surgical procedures showed normal histological image of the gingiva (Figure 1a). On histological preparations of the control gingiva of a false treatment type, 1 h and a day after the treatment mild inflammatory reaction was visible (Figure 1b). After 7 and 30

Table 2

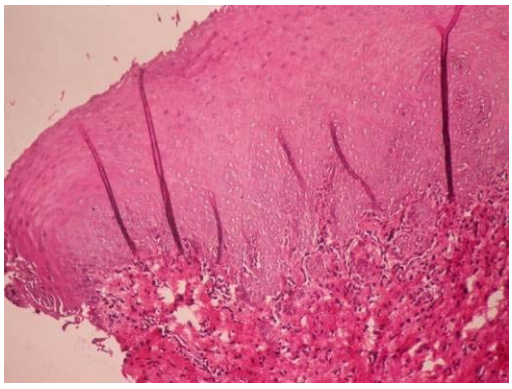
Experimental design of the study

Rabitt	Treatment		Observation period [hour(s) - h; day(s) - d]
	right incisor (upper – u; lower - l)	left incisor (upper – u; lower - l)	
1st	NC(u)	TA (u)	1d
	FT (l)	TA (l)	1h
2nd	NC(u)	TA (u)	1h
	FT (l)	TA (l)	1d
3rd	NC(u)	TA (u)	1d
	FT (l)	TA (l)	1h
4th	NC(u)	TA (u)	1h
	FT (l)	TA (l)	1d
5th	NC(u)	TA (u)	30d
	FT (l)	TA (l)	7d
6th	NC(u)	TA (u)	7d
	FT (l)	TA (l)	30d
7th	NC(u)	TA (u)	30d
	FT (l)	TA (l)	7d
8th	NC(u)	TA (u)	7d
	FT (l)	TA (l)	30d

TA - tested retraction agent; NC - negative control (intact control); FT – false treatment.



a)



b)

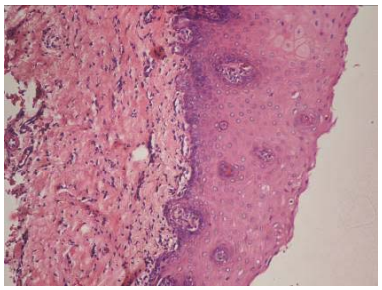
Fig. 1 – a) The tissue samples of the intact controls (the negative controls) had normal amount of collagen fibres; b) Negative control preparations after one day biopsy showed mild focal inflammatory infiltrates that were later replaced with healthy connective tissue (HE, ×200).

days from the false treatment examined gingivae were not histologically different from the negative controls.

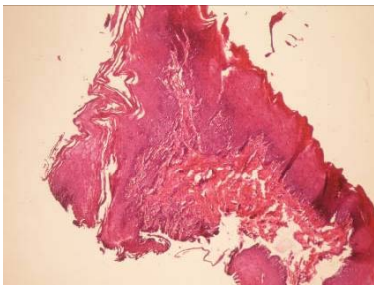
The gingival samples treated with different retraction agents showed different degrees of inflammatory reaction. One hour after removal of a retraction cord, gingival tissue showed slight inflammatory changes compared to the controls. All the tissue samples had foci of inflammatory infiltrate with a reduced amount of collagen fibers (Figure 2).

A day after removal of a retraction cord, inflammatory infiltration was more prominent in the tissue samples treated with retraction agents in relation to those treated with tetrahydrozoline (Visine®) (Figure 3).

After a 7 day observation period, the tissue samples treated with retraction agents showed signs of extensive acute inflammation. More intense degradation of collagen fibers was observed after application of epinephrine-based



a)



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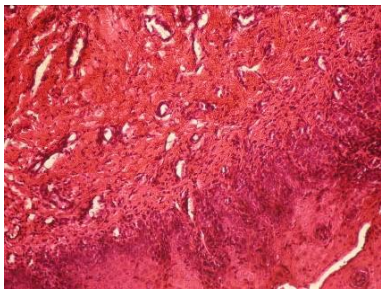

c)

Fig. 2 – Histopathological findings after a 1-hour biopsy.

a) Retrargin® (25% aluminium chloride hexohydrate); b) Surgident® (8% epinephrine-HCl); c) Visine® (0.05% tetrohydrozoline hidrochloride). All tissue samples presented foci of inflammatory infiltrate with reduced amounts of collagen fibres (HE ×100).

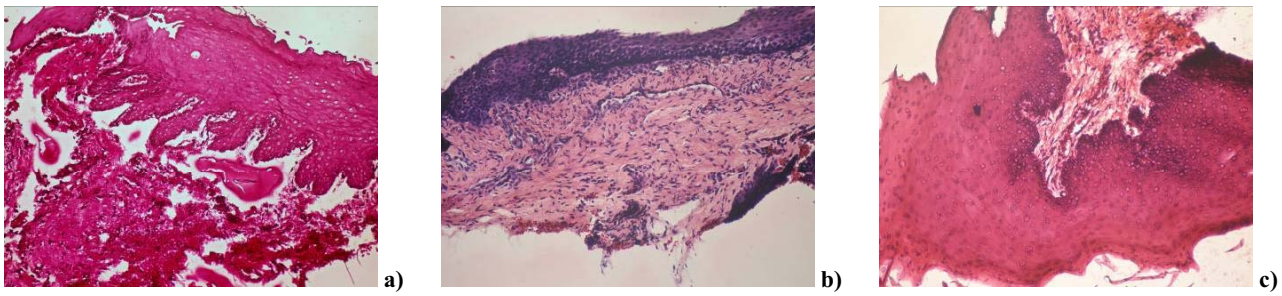

Fig. 3 – Histopathological findings after a 1-day biopsy (HE, ×100).
a) Retrargin® (25% aluminium chloride hexahydrate): tissue edema, with focal inflammatory infiltrate, b) Surgident® (8% epinephrine-HCl): tissue showed strong acute inflammatory response: c) Visine® (0.05% tetrahydrozoline hidrochloride): there was a mild inflammatory reaction, but noticeably lower than in the tissue after the removal of retraction cords.

agent. Tetrahydrozoline-based preparation showed the least inflammatory effect in this case, where tissue fibrosis was observed on a histopathological preparation (Figure 4).

With increasing duration of observation period, there occurred tissue fibrosis, and inflammation became chronic. The newly formed fibrous tissue was the sign of defect reparation in the treated tissue. Complete reparation occurred only in case of tetrahydrozoline application (Figure 5).

Figure 6 shows different amounts of collagen in the tissue structure observed under the polarization microscope after a 7-day observation period. A small amount of collagen observed after application of retraction agents was the sign of more intense acute inflammatory response.

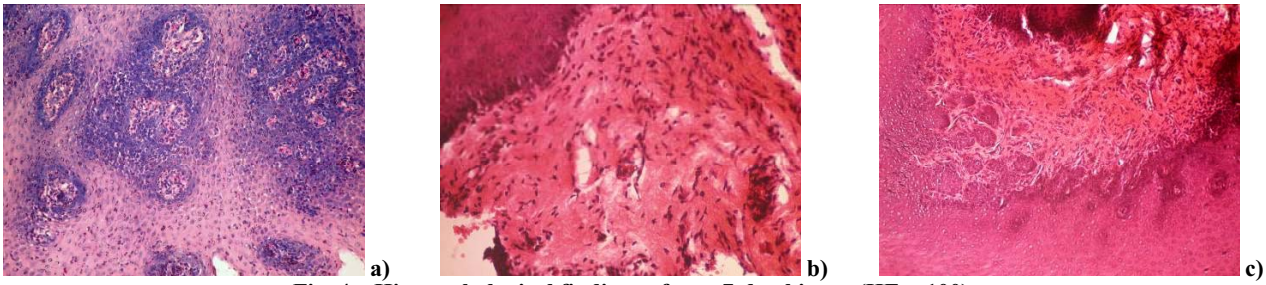

Fig. 4 – Histopathological findings after a 7-day biopsy (HE, ×100).
a) Gingival liquid: tissue samples after the removal of retraction cords with aluminum chloride showed signs of extensive inflammatory reactions; b) Surgident® (8% epinephrine-HCl): inflammatory response and the reduced amount of collagen fibers of small-scale than that presented in Fig. – 4a); c) Visine® (0.05% tetrahydrozoline hidrochloride): tissue fibrosis.

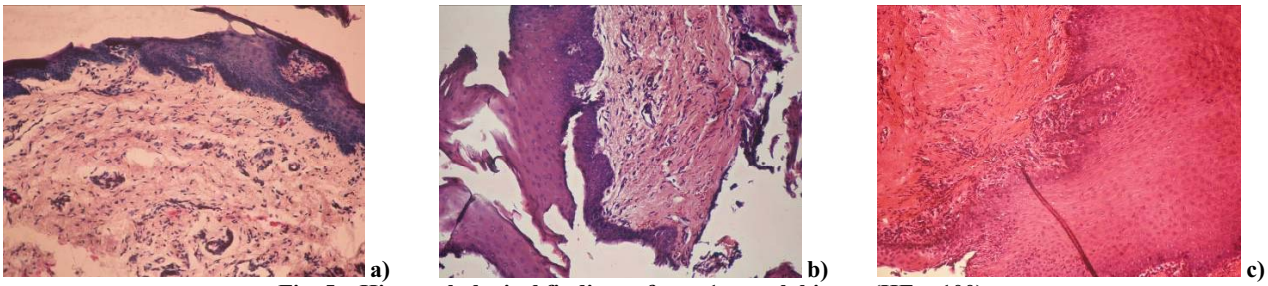

Fig. 5 – Histopathological findings after a 1-month biopsy (HE, ×100).
a) Retrargin® (25% aluminium chloride hexahydrate) and b) Surgident® (8% epinephrine-HCl): tissue preparations showed a reduced amount of collagen fibers and less focal inflammatory infiltrates, c) Visine® (0.05% tetrahydrozoline hidrochloride): a month after the removal of terahydrozoline, there was complete reparation of gingival tissue.

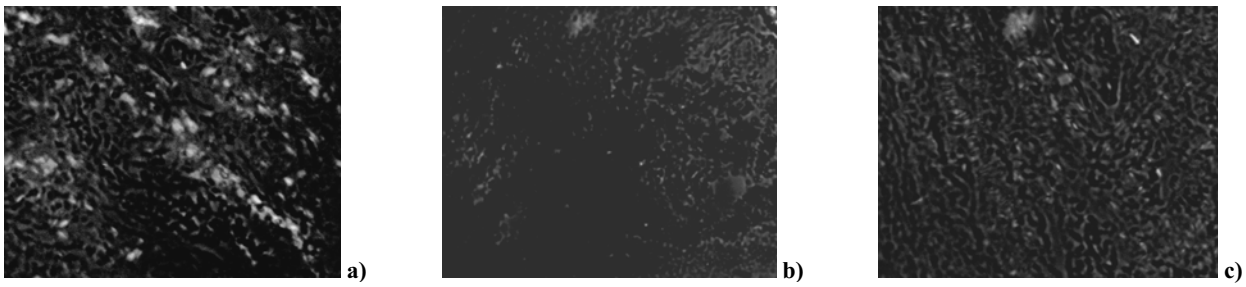

Fig. 6 – Amount of collagen fibers in the gingival tissue 7 days after the application of retraction agents (magnification ×100).
a) normal amount of collagen in the negative controls; b) reduced amount of collagen in the gingival tissue treated with Retrargin® (25% aluminium chloride hexahydrate); c) reduced amound of collagen in the gingival tissue treated with Visine® (0.05% tetrahydrozoline hidrochloride).

Discussion

In order to make an adequate impression of the marginal line area located in or below the gingival level it is necessary to dilate and dry the gingival sulcus. The consequent ischaemia is reversible and is accompanied by reactive hyperemia limited to a 17-minute period upon cord removal [8]. Prolonged ischaemia might lead to tissue damage and necrosis. Changes that occur after retraction procedure usually last from 1 to 2 weeks [10]. Still, infection or serious tissue damage may develop within this period [5]. Changes occur at the junction line of gingiva and connective tissue and may result in periodontitis, apical migration of epithelial junction and alteration of cement surface. After a period of tissue reparation clinically acceptable apical migration of marginal gingiva must not exceed 0.1 mm [4, 11].

Jokstad [12] has shown that the retraction effect of epinephrine- and aluminum-salt-based astringents is almost equal. On the other hand, there is a wide range of adverse systemic reactions to absorption of epinephrine, which significantly reduces its indication area. Therapeutic effect of epinephrine is vasoconstriction of blood vessels, leading to increased blood pressure and heart rate. The risk increases if epinephrine in retraction agent is combined with local anaesthetic, endogenous secretion in a stressful situation, or at greater damage of gingival tissue during tooth preparation [13]. In this study, retraction agents were administered to healthy gingival sulci, without previous tooth preparation, so as not to damage the tissue during preparation and thus jeopardize the objectivity of the results. Retraction was the result of local absorption of a retraction agent and the degree of resorption depended on the degree of tissue damage as well [14].

Previous studies have shown that retraction agents damage epithelium, sulcus epithelium as well as connective tissue *in vitro* and *in vivo* conditions [6, 15]. Changes in the periodontal tissue may be the result of mechanical damage of epithelium during application of retraction cord, but are more often related to the effect of the applied retraction agent. From the clinical point of view, the use of retraction cord without retraction agent indicates lower therapeutic effect [11].

The obtained results show that careful application of retraction cord cause no inflammatory changes in gingival tissue. The study results indicate a reversible damage to gingival tissue as a result of local application of aluminum-chloride- and epinephrine-based retraction agents. There were no significant changes in tissue structure 1 h after retraction agents removal. However, their use led to acute inflammatory response after an observation period of one and seven days. After thirty days reparation of damaged tissue was observed. These results are consistent with the findings of Harrison [16] and Ramadan et al. [17].

Tetrahydrozoline belongs to the group of sympathomimetic vasoconstrictors or α-adrenergic agonists and is commercially available as nasal and olfactory decongestants. Systemic reactions to the use of these products are very rare, given that the maximum recommended doses are significantly higher than those required for effective gingival retraction [2]. Studies by Bowles et al. [2] showed a satisfactory clinical effect of tetrahydrozoline, strong local vasoconstrictive effect and absence of systemic reactions. Clinical study conducted by Tardy et al. [18] demonstrated greater retraction efficiency of tetrahydrozoline in relation to epinephrine without adverse effects.

An *in vitro* study by Kopač et al. [19, 20] found significantly lower damage of cell cultures treated by tetrahydrozoline compared to aluminum chloride. Retraction agents represent acidic solutions with pH values from 0.8 to 3, the parameter which is considered to be major cause of periodontal tissue damage [21]. Conversely, pH value of tetrahydrozoline is 5.6, so it is considered biologically acceptable from that point of view [20]. An *in vitro* study of Nowakowska et al. [22] showed high cell viability values of human gingival fibroblasts after treatment with tetrahydrozoline-HCl based gels. On the other hand, the authors demonstrated cytotoxic activity of astringent retraction agents [23].

Inflammatory changes occurred as the result of application of tetrahydrozoline were of significantly lower intensity compared to the retraction agents based on aluminum chloride and epinephrine, and resulted in a complete tissue reparation after a 1-month observation period. Tetrahydrozoline proved to be biologically acceptable in relation to the investigated retraction agents. As biocompatibility is considered to be an essential feature of dental materials, clinical use of terahydrozoline is recommended [24].

Conclusion

All the examined retraction agents led to an acute inflammatory infiltration of gingival tissue in rabbits, which eventually became chronic. The inflammatory response to the administered tetrahydrozoline was significantly lower with complete reparation of tissue. Taking this fact into account it is recommended as a potential retraction agent.

Acknowledgment

This study was supported by the Project N° 41017 founded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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Received on May 24, 2012.
Revised on October 18, 2012.
Accepted on November 14, 2012.

ORIGINAL ARTICLE

UDC: 616.348/.351-006
DOI: 10.2298/VSP121221024K

Expression of matrix metalloproteinases 2, 7 and 9 in patients with colorectal cancer

Ekspresija matriks metaloproteinaza 2, 7 i 9 kod bolesnika sa kolorektalnim karcinomom

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Abstract

Background/Aim. Matrix metalloproteinases (MMPs) are perceived to play a key role in tumor invasion and metastasis by their capacity to degrade basement membranes and extracellular matrix proteins. The aim of this study was to investigate the expressions of MMP-2, MMP-7 and MMP-9 in tumor tissue and their relation to clinicopathologic features in patients with colorectal cancer. **Methods.** Specimens of resected colorectal cancer and surrounding normal tissue of 82 patients were immunohistochemically stained for MMP-2, MMP-7 and MMP-9. The results of immunohistochemical expression of MMPs were correlated with some clinical and pathologic parameters. **Results.** Immunohistochemical expression of MMP-2 was more frequent in the patients with higher preoperative serum levels of carcinoembryonic antigen (CEA) (p = 0.047), MMP-2 (p = 0.018), MMP-9 (p = 0.036) and in those with lymph node metastasis (p=0.018) and the advanced stage of the disease (p = 0.046). Expression of MMP-7 was more frequent in the patients with elevated preoperative serum levels of: CEA (p = 0.012), MMP-7 (p = 0.036), MMP-9 (p = 0.023) and with deeply invasive neoplasms (p = 0.027). MMP-9 cell expression was in a positive correlation with elevated preoperative serum levels of: CEA (p = 0.013), MMP-2 (p = 0.012), MMP-9 (p = 0.018) and depth of CRC invasion, *ie* T-parameter (p = 0.027). **Conclusion.** Immunohistochemical expression of MMPs is a useful indicator of the disease development and progression in patients with colorectal cancer.

Key words:
colorectal neoplasms; matrix metalloproteinases; neoplasm staging; immunohistochemistry; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Smatra se da matriks metaloproteinaze (MMPs) igraju ključnu ulogu u procesima tumorske invazije i metastaziranja preko njihove sposobnosti za degradaciju bazalnih membrana i proteina ekstracelularnog matriksa. Cilj rada bio je ispitati ekspresiju MMP-2, MMP-7 i MMP-9 u tumorskom tkivu i njihovu povezanost sa kliničkopatološkim karakteristikama bolesnika sa kolorektalnim karcinomom. **Metode.** Tkivni uzorci reseciranih kolorektalnih karcinoma i opkružujuće normalno tkivo 82 bolesnika sa kolorektralnim karcinomom bili su imunohistohemijski obojeni za MMP-2, MMP-7 i MMP-9. Rezultati imunohistohemijske ekspresije MMPs korelirani su sa pojedinim kliničkim i patološkim parametrima. **Rezultati.** Imunohistohemijska ekspresija MMP-2 bila je mnogo češća kod bolesnika sa većim preoperatvinim nivoima CEA u serumu (p = 0.047), predoperativnim nivoima MMP-2 (p = 0.018) i MMP-9 (p = 0.036) u serumu, kod bolesnika sa metastazama u limfnim žlezdama (p = 0.018) i sa uznapredovalim stadijumom bolesti (p = 0.046). Ekspresija MMP-7 bila je mnogo češća kod bolesnika sa povišenim preoperativnim serumskim nivoima CEA (p = 0.012), MMP-7 (p = 0.036) i MMP-9 (p = 0.023) i kod bolesnika sa dubokom invazijom neoplazme (p = 0.027). Ćelijska ekspresija MMP-9 bila je u pozitivnoj korelaciji sa povišenim preoperativnim serumskim nivoima CEA (p = 0.013), MMP-2 (p = 0.012), MMP-9 (p = 0.018) i sa dubinom invazije kolorektalnog karcinoma, tj. parametra T (p = 0.027). **Zaključak.** Imunohistohemijska ekspresija MMPs korisni je indikator razvoja i napredovanja bolesti kod bolesnika sa kolorektalnim karcinomom.

Ključne reči:
kolorektalne neoplazme; matriks metaloproteinaze; neoplazme, određivanje stadijuma; imunohistohemija; osetljivost i specifičnost.

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Introduction

Colorectal cancer (CRC) is one of the most common malignant neoplasms in developed countries. Being the third most common malignant disease CRC accounts for an estimated 570,000 new cases per year and it is the second most common cause of death in the Western-European countries and eight in the developing countries [1]. CRC prognosis predominantly depends on the disease stage, but new prognostic factors are being investigated in order to determine disease progression and outcome in patients, as well as postsurgical pharmacology treatment [2].

Matrix metalloproteinases (MMPs) play an important role in several physiological and pathologic processes such as tissue remodeling, wound healing, angiogenesis, morphogenesis of organs, embryonic development, leukocyte migration and tumour invasion and metastasis. MMPs are a multigene family of structurally similar proteolytic enzymes, that is, zinc-dependent endopeptidases, which have the capacity to degrade virtually every component of the extracellular matrix. It is thought that tumor cells overexpress proteases and induce expression of enzymes in the neighboring stromal cells in order to degrade the basement membrane and invade the surrounding tissue [3].

Expression of MMPs in tumor tissue is regulated by the growth factor and cytokines that are secreted by tumour cells, stromal cells and tumour infiltrating inflammatory cells.

An elevated MMPs activity and their overexpression have been determined in several malignant neoplasms such as lung cancer, pancreatic cancer, ovarian cancer, prostate cancer, breast cancer and brain cancer, and a correlation with the tumor aggressiveness and its malignancy potential has been detected [4, 5]. Earlier studies presented contradictory results related to the expression of the most commonly associated MMP-2 (gelatinase A), MMP-7 (matrilysin) and MMP-9 (gelatinase B) with prognosis in CRC patients, which was the motive to conduct our study [6–8].

Among MMPs, matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9), as members of gelatinases, play important roles in the migration of malignant cells, because of their ability to degrade type IV collagen [9]. The mechanisms of activation of these enzymes are different. MMP-9 modulates permeability of the vascular endothelium, whereas MMP-2 promotes cleavage of extracellular matrix proteins and is intensively expressed by tumor and stromal components of cancer [10].

Matrix metalloproteinase 7 (MMP-7) or matrilysin, as a member of stromelysins is able to induce cell apoptotic impairment. Matrilysin can regulate angiogenesis either by inducing a direct proliferative effect on vascular endothelial cells or producing angiogenesis inhibitors (angiostatin, endostatin and neostatin-7) or enriching the variety of angiogenesis mediators, such as the soluble vascular endothelial growth factor (sVEGF)[11]. It degrades type IV and X collagen, elastin, fibronectin, laminin, osteopontin, proteoglycans, as well as numerous others substrates [12].

Increased levels of MMPs in tumor tissues or in blood circulation have been found to correlate with many cancers, including colorectal cancer (CRC). Several previous studies have shown that MMPs may play an important role as an indicator of CRC occurring and its progression [13, 14].

The aim of this study was to investigate the expressions of MMP-2, MMP-7 and MMP-9 and their relation to clinicopathologic parameters in CRC patients.

Methods

The study included a total of 82 previously untreated CRC patients, 30 (36.58%) were females and 52 (63.41%) males, ages ranging from 43 to 75 years, the mean age of 67.85 years (SD ± 9.67) with operable CRC, without detectable distant metastases, who respected the medical instructions and were available for follow-up. All the patients underwent surgical resection of the primary neoplasm at the University Clinic for Abdominal Surgery in Skopje in the period of 2 years (2007–2009).

Blood samples from all the patients were drawn before surgical treatment in order to examine CEA, CA 19.9, MMP-2, MMP-7 and MMP-9 serum levels. None of the CRC patients had received chemotherapy before blood sample collection. To standardize clotting conditions, all sera were separated within 1 h after blood collection, aliquoted and stored at -80°C until assayed.

Serum levels of CEA and CA 19.9 were determined using an enzyme immunoassay (EIA) (Monobind Inc., USA) according to the manufacturer's instructions. Serum levels of MMP-2, MMP-7 and MMP-9 were determined using a quantitative solid phase sandwich enzyme linked immuno sorbent assay (ELISA, R&D Systems, USA) according to the manufacturer's instructions. MMP-2, MMP-7 and MMP-9 technique can detect both pro- and active forms of recombinant human MMP-2, MMP-7 and MMP-9. High concentrations of MMP-2, MMP-7 and MMP-9 were diluted with a calibrator, to produce samples with a values within the dynamic range of the assay.

The resected specimens were sent to the Institute of Pathology, Medical Faculty in Skopje, where the pathologic stage of the disease in each and every patient was determined according to the Tumor Nodes Metastasis (TNM) classification of American Joint Committee on Cancer (AJCC) (2010).

Immunohistochemical staining

Tissue sections for immunohistochemistry were taken from tumor tissue of the invasive neoplasm front and of the peritumoral tissue without obvious macroscopic changes; they were fixed in formalin, embedded in paraffin and cut at 5–7μ and were primarily stained with hematoxylin eosin.

For immunohistochemical staining monoclonal antihuman MMP antibodies 2, 7 and 9; mouse IgG, clone 36006.211, 6A4 and 36020.111, respectively, Cat.No MAB902, MAB907, MAB936, R&D Systems, Inc. and polyclonal rabbit anti-human matrix metaloproteinase 9, code AO150 Dako were used.

Then the sections were deparaffinized in xylene, rehydrated through a series of graded alcohol solutions and pretreated for antigen retrieval in 10 mM citrate buffer (pH 6.0) in a microwave oven for 15 min at 700 W, and left in the buffer to cool at room temperature.

Endogenous peroxidase activity was suppressed with a solution of peroxidase-blocking reagent (DakoCytomatin, Germany) for 10 min, and nonspecific antibody binding was blocked with protein block serum-free (DakoCytomation, Germany) incubation for 10 min. The sections were incubated with the primary antibodies, diluted with antibody diluents (DakoCytomation, MMP-2 1:200, MMP-7 1:30, MMP-9 1:100) for two hours in a wet chamber at room temperature. For subsequent staining EnVision+two step visualization technique (Dako, Germany) was used with diaminobenzidine (DAB) as a chromogene, incubated for 10 min, and hematoxylin for counterstaining. Omission of the primary antibody served as negative control and carcinoma tissue with high expression of relevant proteins served as positive control.

Immunohistochemical expression of MMP-2, MMP-7 and MMP-9 was determined semi-quantitatively by defining the signal intensity and quantity of immunohistochemically stained cells.

Staining was considered to be negative when 0–10% of tumor epithelial cells were stained, and staining was considered to be positive when > 10% of tumor epithelial cells were stained.

The intensity of the staining pattern was scored to be weak (+), moderate (++) and strong (+++).

Stromal cells positivity was considered to be weak (+) if 1–2 stromal cells were stained, moderate (++) if groups of 3–5 cells were stained and strong (+++) if there were diffusely stained cells or groups of more than 5 cells. The intensity of staining was determined in the same manner as in the epithelial cells.

The cell quantity was determined in the invasive front of the neoplasm.

All specimens were independently evaluated by two pathologists.

Statistical analysis

Statistical analysis was done by applying the Pearson's χ^2test, Fisher's exact test, Mann-Whitney's *U*-test, analysis of variance and Kolmogorov-Smirnov test. Differences were considered statistically significant for *p* values < 0.05.

Results

The localization of analyzed cancers and number of cases are listed in Table 1.

There have been 17 (20.73%) patients in stage I of the disease, 40 (48.78%) patients in stage II and 25 (30.48%) patients in stage III. Lymph node metastases were substantiated in 25 (30.48%) patients and were not find in 57 (69.51%) patients with different pT category (Table 2). The majority of patients were with pT3N0M0 (26.82%), *ie* patients in stage II A of the disease, and the smallest number of patients were with pT4aN1M0 (4.87%), *ie* patients in stage III B of the disease.

Immunohistochemical staining with anti-MMP-2

Positive immunoreactivity in tumor cells for MMP-2 was detected in 19/82 cases (23.17%), and in stromal cells in 27/82 cases (32.92%).

Table 1

Localization and the number of colorectal cancer cases

Localization of cancers	Cancer cases	
	n	%
Rectum	13	15.85
Rectosigmoideum	17	20.7
Sigmoideum	14	17.07
Colon descendens	10	12.19
Colon transversum	3	3.65
Flexura lienalis	7	8.53
Flexura hepatica	3	3.65
Colon ascendens	3	3.65
Coecum	12	14.63
Total	82	100

Table 2

Tumor nodes metastasis (TNM) staging of the disease in colorectal cancer patients according to the American Joint Committee on Cancer

TNM stage	pTNM	Patients	
		n	%
I	pT1 N0 M0	8	20.73
	pT2 N0 M0	9	
II	pT3 N0 M0	22	48.78
	pT4a N0 M0	18	
III	pT3 N1b M0	7	30.48
	pT3 N2a M0	9	
	pT4a N1b M0	4	
	pT4a N2b M0	5	

Cytoplasmic positive immunoreactivity in tumor cells of weak intensity (+) was detected in (84.21%) 16 cases, and of moderate intensity (++) in 3 (15.78%) cases.

The signal intensity of stained stromal cells in all the evaluated cases was assessed to be strong (+++).

Elongated fibroblastoid types of cells were positively stained in the stroma. There was no specific arrangement of stromal positive cells, except for the number of positively stained cells that was semi-quantitatively evaluated, which was larger in the invasive front of the neoplasm (+++) than in the neoplastic stroma in other regions. There was also a larger number of MMP-2 positive cells in the regions with more distinct inflammatory reaction to the neoplastic process.

Sections from the tumor neighboring tissue showed no immunoreactivity with anti-MMP-2 antibody either in the mucosal epithelial cells or in lamina propria.

Immunohistochemical staining with anti-MMP-7

Staining with anti-MMP-7 antigen showed a weak cytoplasmic reactivity (+) with tumor epithelial cells in 32 (39.02%) cases whereas positive stromal staining was detected in 4 (4.86%) cases alone in the regions of inflammatory reaction.

Sections from the tumor neighboring tissue showed no immunoreactivity with anti-MMP-7 antibody neither in the mucosal epithelial cells nor in lamina propria (Figure 1).

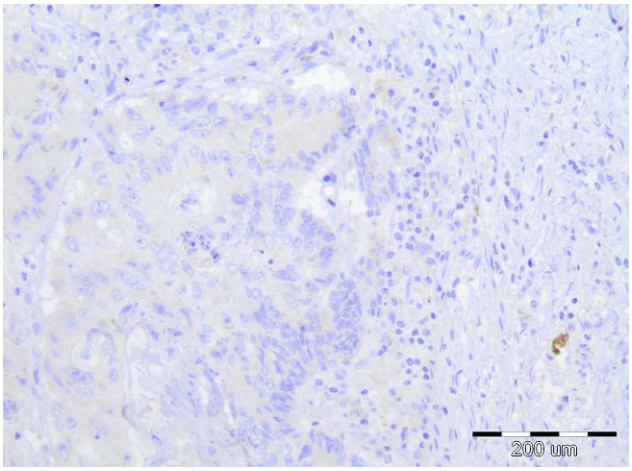

Fig. 1 – Weak staining with anti-MMP-7 for tumor cell components (+) and negative staining for stromal cells (-) with exception of 2 stromal cells (< 10%) (MMP-7 IHH, 10 × 40).

Immunohistochemical staining with anti-MMP-9

Staining with anti-MMP-9 showed positive immunoreactivity of weak intensity in tumor epithelial cells in 37 cases (45.12%), stromal positivity in fibroblastoid cells in 1 case (1.21%) and positivity of inflammatory cells in 79 cases (96.34%). There was a strong staining intensity of inflammatory cells in all specimens. Inflammatory cells were grouped in the invasive front of the neoplasm, while there were few and scattered in the other regions of the tumor stroma. Macrophages showed the most intense staining (Figure 2).

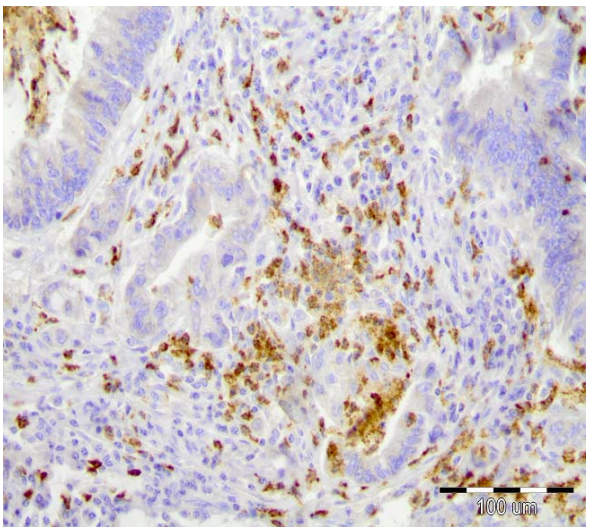

Fig. 2 – Immunohistochemical staining with MMP-9. A weak signal of neoplastic cells (+) and strong signal of stromal inflammatory cells (+++); (MMP-9 IHH, 10 × 20).

Sections from the tumor neighboring tissue showed no immunoreactivity with anti-MMP-9 antibody either in the mucosal epithelial cells nor in lamina propria, except in the inflammatory cells if present in the specimens (Figures 3 and 4).

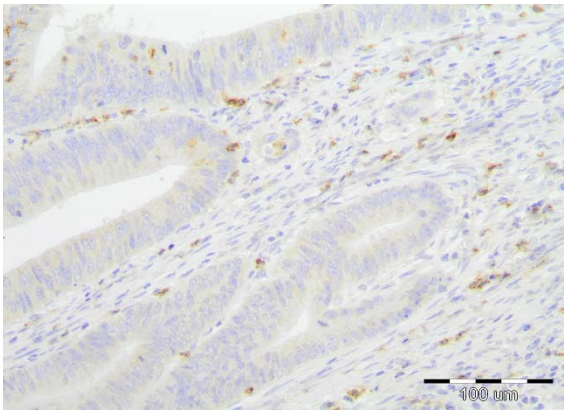

Fig. 3 – Immunohistochemical staining with MMP-9. A weak signal of all epithelial cells (+++) and a strong signal of stromal inflammatory cells (+++); (MMP-9 IHH, 10 × 20).

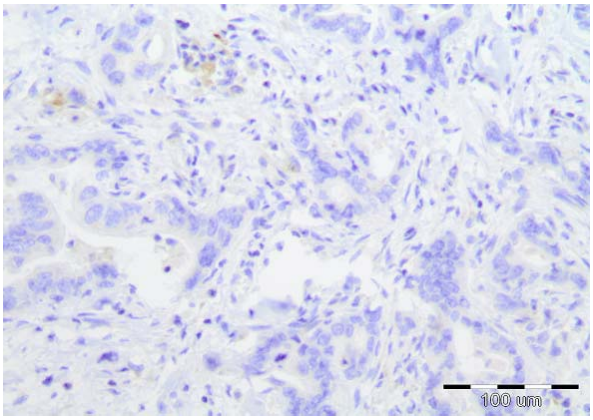

Fig. 4 – Immunohistochemical staining with MMP-9. Negative staining of neoplastic epithelium and negative staining of stromal inflammatory cells (< 10%) (MMP-9 IHH, 10 × 20).

Immunohistochemical staining using antibodies against MMP-2, MMP-7 and MMP-9 showed a statistical overexpression in the neoplastic tissue when compared with the neighboring normal tissue ($p < 0.001$).

The correlations between clinicopathologic parameters and expression of MMPs are presented in Table 3.

Table 3

Correlations between clinicopathological features and the expression of MMP-2, MMP-7 and MMP-9

MMPs expression	MMP-2 Cell		MMP-2 Stromal		MMP-7 Cell		MMP-7 Stromal		MMP-9 Cell		MMP-9 Stromal	
	Positive n = 19	*p*	Positive n = 27	*p*	Positive n = 32	*p*	Positive n = 4	*p*	Positive n = 37	*p*	Positive n = 79	*p*
CEA (ng/mL)												
< 5	4		19		27		3		21		59	
> 5	15	0.047	8	ns	7	0.012	1	ns	17	0.013	20	ns
CA19.9 (U/mL)												
< 37	5		17		21	ns	2	ns	19	ns	57	ns
> 37	14	ns	10	ns	11		2		18		22	
MMP-2 (ng/mL)												
< 200	2		19		18		3		22		45	
> 200	17	0.018	8	0.021	14	ns	1	ns	16	ns	32	0.012
MMP-7 (ng/mL)												
< 2,3	6		13		21		3		18		41	
> 2,3	13	ns	14	ns	11	0.036	1	0.047	19	ns	38	ns
MMP-9 (ng/mL)												
< 400	3		16		16		4		29		43	
> 400	12	0.036	11	ns	18	0.023	4	ns	8	0.018	36	ns
pT1	2		9		7		0		6		14	
pT2	5	ns	8	ns	11	0.039	2	ns	10	0.027	24	0.027
pT3	12		10		14		2		21		41	
Limph nodes												
present	15	0.018	17	ns	17	ns	2	ns	17	ns	43	ns
absent	4		10		15		2		21		36	
Stage I	4		6		9		0		9		18	
Stage II	5	ns	6	0.046	10	ns	2	ns	11	ns	29	ns
StageIII	9		15		13		2		17		32	

ns – statistically not significant; pT– pathological tumor (from pTNM classification); N – Limph nodes (from pTNM classification)

MMP-2 cell expression was in a significant positive correlation with serum levels of CEA and MMP-2 preoperatively in the CRC patients, and serum levels of MMP-9 and metastasis in the lymph nodes. Stromal positivity to MMP-2 was in a positive correlation with serum levels of MMP-2 and disease stage.

MMP-7 cell expression was in a significant positive correlation with serum levels of CEA, MMP-7 and MMP-9 and depth of tumor invasion. No significant correlations were obtained between stromal expression in MMP-7 and any of the examined parameters.

MMP-9 cell expression was in a positive correlation with serum levels of CEA and MMP-9 and depth of CRC invasion, *ie* T-parameter. Stromal positivity to MMP-9 was in a significant positive correlation with serum levels of MMP-9 and depth of tumor invasion.

Stromal expression of MMP-2 was also in a significant positive correlation with the depth of invasion.

Cell expression of the examined enzymes, MMP-2, MMP-7 and MMP-9 was in a positive correlation with serum levels of CEA, MMP-2 and MMP-9. Expression of MMP-7 and MMP-9 was in a significant positive correlation with depth of invasion, MMP-2 was in a significant correlation with the presence of metastasis in the lymph nodes and MMP-7 was in a significant correlation with stage of the disease (Table 3).

Discussion

Prognosis of newly diagnosed CRC cases is predominantly based on the disease stage defined according to the International Union Against Cancer (UICC-TNM) and American Joint Committee on Cancer (AJCC, 2010), that is, on the local spread of the disease, lymph nodal status and the presence or absence of distant metastasis.

In spite of the advancement in surgical techniques and pharmacological strategies of adjuvant and neoadjuvant therapy, the 5-year survival in CRC patients is estimated to be from 90% to 10% depending on tumour progression [15].

However, the already accepted fact that CRC is a heterogenic, multifactorial disease has been shown by the fact that histologically identical tumors might have different prognosis and different therapy response [16].

New methods and possibilities are being investigated that might find practical application in anticipation of the disease course and outcome [17–20].

Invasion and metastasis are major biological features of malignant neoplasms and they are main cause for morbidity and mortality related to malignant diseases [21, 22].

In our study we made an analysis of immunohistochemical staining of MMP-2, -7 and -9 in cancer tissue specimens and correlated the findings with serum levels of CEA, MMP-2,-7 and -9 and with the local tumor invasion and the presence of metastases in 82 patients with colorectal cancer. We found out that positive immunohistochemical staining of MMPs is in correlation with preoperative serum level of CEA, MMP-2, MMP-9, depth of invasion, limph node metastasis and disease stage.

Numerous studies have proved that the MMP family plays an essential role in malignant tumor growth. Early experimental and morphological studies have demonstrated that carcinoma cells have immunohistochemical expression of MMP-2, showing the ability of carcinoma cells to synthesize MMPs [23,24].

Later, *in situ* hybridization supported the findings that stromal tumor cells *in vivo* create mRNA for MMP-2 [25]. It was further confirmed that MMP-9 is produced both by cancer cells and stromal cells and that stromal cells matrix proteins, such as laminin, have an impact on MMPs secretion [26].

Contemporary researches more often emphasize MMPs to be prognostic factors for several types of malignant tumors, including CRC [27].

Diverse results have been obtained from numerous examinations performed to determine the significance of MMPs in the diagnosing of malignancies and to determine their influence on the outcome [6].

In 1998, in order to determine active and inactive MMP-2 and MMP-9 expression, Pearsons et al. [28] examined on 53 colorectal carcinomas, 15 colorectal adenomas and 15 gastric carcinomas upon which they determined that in both colorectal and gastric carcinomas there was overexpression of the two enzymes [28].

Tutton et al. [29] made an examination in order to determine MMP-2 and MMP-9 distribution in CRC patients in comparison to the levels of the two enzymes in patients plasma and the changes that occur in plasma after resection, in order to determine whether plasma levels were the consequence of clinical staging and the development of the disease. That examination determined that the MMP-2 plasma levels were considerably elevated in patients with CRC, that they considerably decreased after surgical resection of the tumor, and that the MMP-9 serum levels were considerably elevated in all stages of the diseased in patients with CRC, while they decrease after the surgical resection of neoplasm.

On the contrary, in the Ruokolainen's [30] investigation for the prognostic role of MMP-2 and MMP-9 and their tissue inhibitors (TIMP-1 and TIMP-2) in squamous head and neck cancer, was shown that serum MMP-2 immunoreactive protein levels in check-ups of healthy patients were higher than in the patients with cancer, while MMP-9 and TIMP-1 levels were considerably higher in patients with squamous carcinoma. The authors determined an important correlation between the serum levels of MMP-9 and TIMP-1 with immunohistochemical expression of MMP-9 and TIMP-1 from the tumor tissue.

Dragutinović et al. [31] in their study of 32 CRC patients and another 11 controls using immunohistochemistry and CEA serum values, CA 19-9 and MMP-2 and 9 determined that there was an important correlation of the MMP values with staging, but not with CEA and CA 19-9 serum values. They concluded that the serum MMP-2 and MMP-9 detection can be a useful tool for identification of the patients with CRC.

Maurel et al. [32], during the investigation of MMP-7 serum levels in 87 check-ups of healthy patients and in 120 patients with CRC in order to determine the serum level prognostic significance of this enzyme report that the patients with advanced cancer have considerably higher average MMP-7 values in comparison to those without metastases and in comparison to the healthy patients check-ups. They have determined that MMP-7 levels are in important correlation also with the shorter survival time, which leads them to the conclusion that elevated MMP-7 serum levels are independent prognostic factor for survival in patients with advanced CRC.

Serum measurements of total MMP-2, MMP-7 and MMP-9 can be considered as an indirect estimation of tumor MMP-2, MMP-7 and MMP-9 expression.

There are studies in which immunohistochemical expression of MMPs are correlated to clinical and pathological parameters with different results.

Kim and Kim [33] in their study from 1999 showed the correlation between the expression of MMP-2 and MMP-9 and angiogenesis in CRC. They presented a positive immunohistochemical staining pattern of strong intensity for MMP-2 in tumor cell cytoplasm at the invasive front of the tumor and they found out that the intensity and distribution of staining were well correlated with the modified Astler-Coller classification. Positive staining for MMP-9 was restricted to cytoplasm of tumor cells and isolated stromal cells. The intensity of cytoplasm staining was not in agreement with the modified Astler-Coller classification, lymph nodal status or depth of invasion. Tumor microvascular density was higher in CRC patients with MMP-2 expression than in those patients where the tumor showed no MMP-9 expression.

An immunohistochemical investigation conducted in 2005 by Li et al. [34] at specimens of colorectal cancer showed that the expression level of MMP-2 was higher in CRC tumor tissues than in normal tissues. The authors showed that the expression level of MMPs was related to depth of invasion, lymph node metastasis and Duke's stage and that the expression of TIMP-2 in tumor tissues was lower than that in normal tissues. They also presented that with the progression of tumor invasion, lymph node metastasis and stage, the expression of TIMP-2 increased, but it never reached the expression of the normal colorectal tissue.

Another similar investigation conducted in the same year showed that MMP-7 expression in tumor tissues was associated with lymph node metastasis and a poor five-year survival, and MMP-9 expression was related to the depth of tumor invasion [35].

The aim of the Schwandner et al. [36] study was to determine the prognostic role of MMPs in CRC. They presented positive staining for MMP-2 both in tumor tissue and in stroma, 35% and 77% respectively, where stormal staining pattern was correlated with the depth of invasion, MMP-7 and TIMP-2 expression. Cytoplasmic staining of neoplastic cells was in correlation with MT1-MMP (membrane-type 1 matrix metalloproteinase) and TIMP-2 expression. The authors of this study found no correlation between immunohistochemical staining pattern for MMP-2 and gender, age, grading, stage, nodal status and preoperative serum CEA level. Regarding staining with anti-MMP-7 the authors found positive expression in tumor epithelial cells, but not in stromal cells in 51% of cases. This staining was in correlation with depth of invasion and TIMP-2 expression.

In comparison to the above reports in our study we found out that positive tissue expression of all the examined

enzymes, MMP-2, MMP-7 and MMP-9, was in a positive correlation with serum levels of CEA, MMP-2 and MMP-9. Expression of MMP-7 and MMP-9 was in a significant correlation with the presence of nodal metastasis, and MMP-7 was in a significant correlation with disease stage.

Conclusion

Our investigation confirmed the presence of MMP-2, MMP-7 and MMP-9 in tumor cells and tumor stroma with significantly more common immunoreactive expression compared to that in normal tissue.

We found out a positive significant correlation of MMP-7 and MMP-9 tissue expression with the depth of invasion, positive correlation of MMP-2 tissue expression with the presence of nodal metastasis, as well as a positive correlation of tissue expression of MMPs with serum levels of CEA, MMP-2 and MMP-9. These correlations indicate that tissue expression of MMPs is a useful indicator of the disease spreading and might be used as a prognostic factor for CRC.

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Received on December 21, 2012.
Revised on February 15, 2013.
Accepted on March 12, 2013.
OnLine-First May, 2013.

GENERAL REVIEW

UDC: 615.322::616.1-084
DOI: 10.2298/VSP1401060K

Evaluating the bioactive effects of flavonoid hesperidin – A new literature data survey

Ocena bioaktivnih efekata flavonoida hesperidina – pregled podataka iz novije literature

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Key words:
hesperidin; antioxidants; cardiovascular diseases; radiation-protective agents; preventive health service.

Ključne reči:
hesperidin; antioksidansi; kardiovaskularne bolesti; radioprotektivi; preventivno-medicinska zaštita.

Introduction

A growing number of epidemiologic studies consistently shows a protective effect of polyphenol-rich foods (fruit, tea, wine, cocoa or chocolate…) against many diseases [1–4]. This evidence is supported by the results of numerous studies conducted in animal models, with nutritionally realistic levels of isolated flavonoids [5, 6], and in humans with flavonoid-rich foods [1–4]. The most famous so-called French paradox, the lack of a positive correlation between a high intake of saturated fat and the occurrence of coronary heart disease is related at least partly to the consumption of red wine [7], which is rich in flavonoids. Due to the variety of pharmacological activities in the human body, flavonoids are referred as "nutraceuticals" [8].

In spite of such extensive number of articles about health benefits of flavonoids, further researches in this field are broadly conducted. In this very moment, on PubMed more than 58,000 references about flavonoids can be found. Since flavonoids are the subject of our longtime research [9], our aim was to give a personal account on the development of this field through a retrospective of some basic data about flavonoids, their pharmacological properties and mechanism of action. In this review, special attention was paid to one of the most promising bioactive bioflavonoids, hesperidin (Hesp).

About flavonoids

Flavonoids (*flavus*, the Latin word for yellow) or bioflavonoids, polyphenolic low weight secondary metabolites, are present in great number of higher plant species, principally placed in fruit bark, seeds or flowers. For the time being, more than 8000 different flavonoids have been identified, and that number constantly increases [10]. Starting from 1936, with the first article about flavonoids bioactivity [11], numerous literature data were published about their structure and characteristics primarily connected to antioxidative activity.

Chemically, flavonoid molecules consist of carbon atoms assembled in two aromatic rings that are connected by a three-carbon "bridge", C_6-C_3-C_6, thus forming a diphenylpropane structure with the central unit being a benzo-γ-pyrone (chromone). Multiple hydroxyl groups, sugar, oxygen, or methyl groups are attached to this core structure. A group of flavonoids is differentiated in several classes according to the degrees of oxidation and unsaturation of the heterocyclic C ring. The major flavonoid classes are: catechins, dihydroxychalcones, chalcons, flavanones, flavanols, flavones, isoflavones, anthocyanidols, aurones and flavonols. Depending on the oxidation state of the heterocyclic ring, flavonoids (in constricted sense) are classified as flavones, flavanonols, flavonols and flavanones [12].

A considerable number of plant medicines contain flavonoids, which have been reported by many authors as having antibacterial [13], anti-inflammatory [14], antiallergic [15], antimutagenic [16], antiviral [17], antineoplastic [18, 19], antithrombotic [20, 21], and coronary heart disease actions [22–25]. Overwhelmingly, pharmacological effects are related to antioxidant activity of flavonoids, arising through their ability to scavenge free radicals. When generated in excess, free radi-

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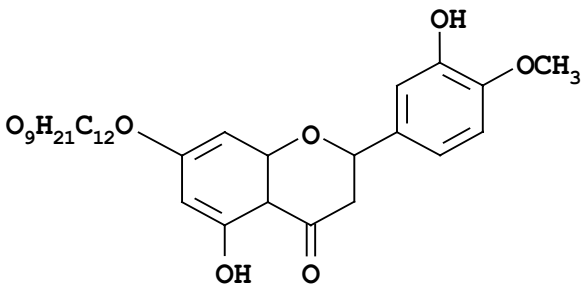
cals can damage biomolecules, and are therefore implicated in the etiology of several diseases and ageing [26]. Radical scavenging by flavonoids occurs *via* electron donation from the free hydroxyls on the flavonoid nucleus, with the formation of a less reactive flavonoid aroxyl radical stabilized by resonance and therefore playing a moderate role in the propagation of the radical-induced damages in biological systems. Antioxidant activity of flavonoids correlates well with their physiological function *in vivo*, because oxidative stress is known to participate in the initial process of different patho-physiological events. Literature data report that flavonoids can prevent injury caused by free radicals by the following mechanisms: direct scavenging of reactive oxygen species (ROS), activation of antioxidant enzymes, metal chelating activity, reduction of α-tocopheryl radicals, inhibition of oxidases, mitigation of oxidative stress caused by nitric oxide, increase in uric acid levels, increase in antioxidant properties of low molecular antioxidants (27 and references therein).

Besides direct free radical scavenging, flavonoids exert antioxidant activity through interactions with the reduced form of transition metals, primarily Cu(I) and Fe(II), as well as Fe(III), which participate in free radical generating reactions [28]. Flavonoids may sequester metal ions by chelation, thereby preventing the metal-mediated generation of free radicals, and accordingly may protect the potential biological targets from oxidative stress. Thus, the overall antioxidant action of flavonoids appears to be a combination of a direct reaction with free radical and chelating metal ions responsible for the production of ROS [29–38]. We also investigated more then 40 metal-flavonoid chelates, as summarized in our review article [9].

Hesperidin

Chemical structure of hesperidin

Hesp, one of the most important flavonoids, is a low molecular weight molecule (molecular weight 610.57 Da), with the bruto formula $C_{28}H_{34}O_{15}$, and belongs to the flavanone class of flavonoids. Chemically, Hesp consists of aglycone (the forms lacking sugar moieties), hesperitin, and sugar rutinoside: hesperetin-7-rutinoside, or IUPAC name: (2*S*)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-[[(2*R*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one. Hesp is represented by the following chemical structure:



Sources of hesperidin

Hesp is the predominant flavonoid in citrus fruits, primarily in sweet orange (in young immature oranges it accounts for up to 14% of the fresh weight of the fruit [39]) and lemon, and consequently in juices made of these citruses. The peel and membranous parts of these fruits have the highest Hesp concentrations, thus hand-squeezed juices contain no detectable traces of Hesp [40]. Commercial juices, on the other hand, are rich in Hesp because the industrial processing of fruits leads to juices being contaminated with the peel constituents. According to the Code of Practice for evaluation of quality and authenticity of fruit and vegetable juices, published by the AIJN – European Fruit Juice Association, special requirements for quality of citrus based juices (oranges, lemon and grapefruit) are that defined the Hesp content be as 250–700 mg/L [41].

Hesperidin pharmacokinetics

Although citrus fruits and juices are widely consumed in the world, little information has been published on flavanone bioavailability in humans. Hesp itself is absorbed from the intestine intact as a glycoside. Its aglycone hesperitin appears in plasma 3 h after ingestion, reaching a peak between 5 and 7 h. The circulating forms of hesperetin are glucuronides (87%) and sulphoglucuronides (13%). For Hesp, urinary excretion is nearly complete 24 h after the orange juice ingestion and does not depend on the dose [42].

Hesperidin bioactivity

Although hesperidin has no usual structural elements to suggest it as a good free radicals capturer and chelator, the ability for chelating metal ions was confirmed in our experiments [43–46]. Several other investigators have examined Hesp antioxidant activity and radical scavenging properties using a variety of assay systems [47, 48]. Jovanovic et al. [49] reports that Hesp reduces superoxide ions in electron transfer plus concerted proton transfer reaction *in vitro*. Further, Hesp was found to be effective in protecting liposomes from UV-irradiation induced peroxidation, probably by scavenging the oxygen free radicals generated by UV-irradiation [50]. Numerous studies confirmed a potent bioactivity of Hesp, such as effects on the vascular system (reduces capillary permeability) [51], anti-inflammatory effects, antioxidant effect, action on enzymes, antimicrobial activity (antibacterial, antifungal, antiviral, ...), anticarcinogenic activity [52], cell aggregation inhibition, antiallergic effects, UV protecting activity, radioprotection, and so on. In the further text, more specific information about Hesp effects on the cardiovascular system are given. Also, the literature describing Hesp as promising protector against ionizing radiation is assembled.

Hesperidin in prevention of (cardio)vascular diseases

In the review article by Garg et al. [53] the pharmacological properties and medicinal uses of Hesp from the available references were widely described. We emphasize some of most essential Hesp activity.

It has been presumed that Hesp could increase capillary resistance thanks to its ability to inhibit hyaluronidase activity. Also, it has been confirmed that Hesp inhibits inflammatory processes in the ischaemia-induced hyperpermeability, characteristic for venous stasis. Hesp capillary antihaemorrhagic activity has also been reported. Thus, Hesp supplementation has been recommended for a wide range of blood vessel disorders, such as fragility and permeability.

Investigations performed on rats showed improved antihypercholesterolaemic activity of Hesp, exhibited as decreasing cholesterol, LDL, total lipids and triglyceride levels, and increased HDL levels. Results of some studies indicate the calcium channel blocker activity of Hesp.

Some studies have demonstrated antihypertensive and diuretic effects of Hesp in rats following oral administration of the drug at the dose of 200 mg/kg body weight, concluding that this hypotensive effect is caused by increased diuresis. On the other hand, it is well established that several flavanones, including Hesp, exhibit influence on enzymes such as protein kinase, lipooxygenase and cyclooxygenase. The antihypertensive activity of Hesp might be due to influences on blood fluidity *via* enzymes. Further, various flavonoids are inhibitors of cyclic-AMP phosphodiesterase, so this activity could be connected to their diuretic effect.

References dating from 2000 to the present time confirm citrus fruit benefits on the vascular system. Thus, the consumption of citrus fruit has been associated with a lower risk of acute coronary events and stroke [54, 55]. From clinical data, citrus juice consumption reduces oxidative DNA damage in blood cells [56] and improves plasma concentrations of inflammation markers and oxidative stress [57–59]. In addition, the consumption of citrus juices improves lipemia in men with previous coronary bypass surgery [60]. Furthermore, in hypertensive subjects, the consumption of flavanone-rich grapefruit juice exerts a significant beneficial effect on blood pressure [61].

The results of a very recent study published in Am J Clin Nutr [62] are in agreement with numerous literature data. In this study, the authors investigated the effect of orange juice and its major flavonoid, Hesp, on microvascular reactivity, blood pressure, and cardiovascular risk biomarkers through both postprandial and chronic intervention studies. During the three 4-week periods, 24 healthy, overweight men (age 50–65) daily consumed 500 mL orange juice, 500 mL control drink plus Hesp (CDH), or 500 mL control drink plus placebo (CDP). All measurements and blood collections were performed in overnight-fasted subjects before and after the 4-week treatment periods. The postprandial study was conducted at the beginning of each experimental period. Diastolic blood pressure was significantly lower after a 4-week consumption of orange juice or CDH than after consumption of CDP, whereas microvascular endothelium-related reactivity was not significantly affected when measured after an overnight fast. However, both orange juice and CDH ingestion significantly improved postprandial microvascular endothelial reactivity compared with CDP when measured at the peak of plasma hesperetin concentration. As a conclusion of this study, Hesp could be linked to the beneficial effect of orange juice.

Although the consumption of Hesp juice rich food has been reported to exert beneficial effects on some intermediate risk factors for CVD, such as LDL cholesterol, blood pressure, and endothelial function [63], only a few clinical trials have dealt with the oral administration of chemically pure Hesp. Thus, the following study with 204 healthy participants evaluated the LDL-C–lowering efficacy of pure Hesp in moderately hypercholesterolemic individuals (serum total cholesterol (TC) concentration of 5.0–8.0 mmol/L) [64]. The study started with a 4 week preintervention period during which the participants had to abstain from consuming citrus fruits and their juices, food supplements containing hesperidin and/or naringin as well as plant sterol/stanol-enriched foods, and other food products or supplements claiming to lower cholesterol. During the 4-week intervention, the participants applied the same dietary restrictions as during the preintervention phase and consumed 4 capsules/d, providing either placebo (cellulose) or the daily dose of 800 mg Hesp or 500 mg naringin. Blood samples to measure serum lipids were taken on 2 consecutive days at the beginning and the end of the intervention phase. The intake of 800 mg/d Hesp administered twice daily with main meals did not lower serum TC and LDL-C in moderately hypercholesterolemic individuals. This outcome suggests that capsule format Hesp exerts no cholesterol-lowering effect in humans.

Hesperidin as radioprotector

During the last several years, some researches were conducted to utilise Hesp as a promising protector against ionizing radiation (IR), since IR, as the part of our environment and in medical treatment, has been established as a strong carcinogen.

IR causes cellular damage which is predominantly mediated through free radicals and resultant ROS [65]. The interaction of IR with water, a major cellular constituent, results in the generation of primary water radical species due to radiolysis of water. These species react with molecules like oxygen-producing secondary radicals (H_2O_2 and O^{2-}), which are highly reactive and could diffuse to vital cellular targets like DNA, proteins, lipids and membrane, ultimately leading to cancer and cell death [66].

Since free radicals play a major role in the initiation and progression of IR induced toxicity, the use of antioxidants either in the diet or as therapeutic agents might offer protection against radiation induced damage. A number of medicinal plants evaluated for their radioprotective efficacy have shown protective effects against the damaging effects of IR. The article of Nambai et al. [67] summeries the effects of various phytochemicals on IR.

The development of effective radioprotectors is of great importance in view of their potential application during both planned radiation exposure (radiotherapy) and unplanned radiation exposure (nuclear accidents, natural background radiation emanating from the earth or other sources). Amifostine, a thiol synthetic compound, is a powerful radioprotective agent (marketed by MedImmune under the trade name Ethyol®). However, due to its side effects and toxicity, the use of this drug should be replaced with less toxic natural compounds [68].

Since much research has been focused on the potential use of flavonoids as free radical scavengers to prevent oxidative damage [69], consequently several researches were conducted to explore Hesp as radioprotector. The experiments were performed either in animals (mouse and rats) or *in vitro* (on human blood), since the study of radioprotective effects is limited due to non-permission to irradiate healthy humans for experimental research. Experiments in animals were performed using different graded doses (mg/kg body weight) of Hesp administered orally *via* intragastric intubations for several days prior to whole body radiation exposure with different doses (1–10 Gy).

Hosseinimehr and Nemati [70] investigated the radioprocective effects of Hesp in mouse bone marrow cells by using the micronucleus test for anticlastogenic and cell proliferation activity. They showed that Hesp has powerful protective effects against DNA damage and on the decline in cell proliferation induced by γ-irradiation in mice by reducing the frequency of the micronuclei.

Pradeep et al. [71] explored the hepatoprotective and antioxidant effects of Hesp against γ-irradiation induced oxidative damage in the liver of male Sprague–Dawley rats. Exposure to γ-irradiation resulted in hepatocellular damage in a dose-dependent manner, featuring a significantly decreased body weight and liver weight and higher levels of serum AST, ALT, ALP, LDH and γ-GT levels and a simultaneous decrease in their levels in the liver tissue. The γ-irradiation induced toxic effects were dramatically and dose-dependently inhibited by Hesp treatment as observed by the restoration in the altered levels of the marker enzymes, lipid peroxidation, enzymatic and non-enzymatic antioxidants.

Kalpana et al. [72] investigated the radioprotective efficacy of Hesp against x-ray radiation induced cellular damage in the liver of Swiss albino mice. The results indicated that radiation-induced decrease in the levels of endogenous antioxidant enzymes and increase in lipid peroxidative index, DNA damage and comet parameters were altered by preadministration with the effective dose of Hesp which restored the antioxidant status to near normal and decreased the levels of lipid peroxidative index, DNA damage and comet parameters, which were confirmed by histopathological examinations.

Besides these studies which have been performed to evaluate natural products for their radioprotective effects in animals, there are a few studies assessing the radioprotective effects of natural origin compounds in human volunteers for reducing the genetic side effects caused by IR. The radioprotective effect of Hesp against genotoxicity induced by γ-irradiation has been investigated *in vivo*/*in vitro* in cultured blood lymphocytes from human volunteers [73, 74]. Peripheral human blood samples were collected predose and after a single oral ingestion of 1,000 mg of Daflon® (a dietary supplement containing 50 mg of Hesp in 500 mg-tablet) corresponding 100 mg of Hesp, then exposed *in vitro* to γ-rays. A significant increase (40%) in the incidence of micronuclei after exposure to γ-irradiation as compared to control unexposed samples was observed. After Daflon® administration, a significant decrease in the incidence of micronuclei was observed in comparison with similarly irradiated lymphocytes collected before administration. These findings suggest a possible application of Daflon® for the protection of human lymphocytes from the genetic damage and side effects induced by γ-irradiation. Similar research was conducted by Kalpana et al. [75] also showing that Hesp offers protection to cultured human peripheral blood lymphocytes against radiation induced cellular damage.

Dosage and administration

In numerous widely spread dietary supplements, Hesp is present as Citrus complex of bioflavonoids, usually in a combination with Vitamin C. In those kinds of products, the content of total bioflavonoids is usually declared by the producer, without the concentration of Hesp alone. For example, in the preparation Vitamin C with citrus bioflavonoids & Rose Hips®, the total citrus flavonoid content, according to the factory declaration, was 751 mg *per* two capsules without declaration for Hesp alone, but we found that each capsule contains 260 mg of Hesp [76]. In combination with a flavone called diosmin, the tablets (trade name Daflon®) for treatment of chronic venous insufficiency and hemorrhoids are broadly available on the European market. A 500-mg dose of this combination product is comprised of 50 mg of Hesp and 450 mg of diosmin. The doses for this type of supplement are usually 500 mg to 2 g daily.

Conclusion

The beneficial effects of flavonoids on human health are universally accepted nowadays. Citrus fruits and juices with Hesp as major flavonoid remain one of the most readily-available dietary sources for their intake. In dietary supplements Hesp is usually present in a combination with vitamin C. Epidemiological studies have shown an inverse association between risk of cardiovascular diseases and intake level of Hesp. Also, some experiments utilise Hesp as a promising protector against IR. Further clinical trials are needed to assess a more precise correlation between the Hesp consumption and human health benefits.

Acknowledgments

We acknowledge the Ministry of Sciences, Education and Technological Development of the Republic of Serbia, under the Projects 172 041 and 172 043, for financial support.

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Received on March 19, 2012.
Revised on August 3, 2012.
Accepted on December 5, 2012.

CURRENT TOPIC

UDC: 618.19-006-08-036:613.71/.72
DOI: 10.2298/VSP1401066B

Physical activity and maximal aerobic capacity in breast cancer survivors – why this is important

Fizička aktivnost i maksimalna aerobna sposobnost kod žena koje su lečene od raka dojke – zašto je to važno

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Key words:
breast neoplasms; motor activity; survival.

Ključne reči:
dojka, neoplazme; fizička aktivnost; preživljavanje.

Introduction

Breast cancer (BC) is the most frequent females' malignant solid tumor in Serbia and the leading cause of death from malignant disease among them [1, 2]. Although the number of new breast cancer patients is constantly rising, thanks to early diagnosis and modern methods of treatment, the number of successfully treated patients is growing, too. Treatment of malignant diseases is associated with numerous adverse effects – physical, mental, emotional and social. Unfortunately, these reactions are often not limited to the period of treatment, but are retained for months, even years after the treatment.

Different modalities of exercise therapy, aerobic training and physical activity in general, have for long been proven as significant factor and strong nonpharmacological tool in the fight against unwanted consequences of malignant disease and its treatment.

During chemotherapy and radiotherapy, the unsuspicious influence of aerobic training on aerobic capacity (VO_2max) and functional ability was demonstrated [3, 4]. It was also shown that aerobic training helps to control the side effects of treatment[5-7], may assist the modulation of level and pattern of daily fatigue [3, 8, 9] even in women with advanced disease [10], and can reduce the symptoms of psychological distress – anxiety, depression and sleep disorders [3, 8, 11]. Resistance exercises, alone or in combination with aerobic training, can improve muscle strength [12–15] and cause the change in body weight and body mass index [16, 17], reduce body fat [13, 15] and increase bone density [18]. It was also shown that such training can enhance adaptive responses to stress and improve quality of life of these persons [12].

Many papers report that aerobic and combined training given after therapy for breast cancer increase aerobic fitness, improve physical fitness [19–24] and increase strength and muscle elasticity [24, 25]. It was shown that such trainings can have a positive effect on fatigue [22, 26, 27], depression [21, 28], and the general quality of life [19–21, 29–31]. Resistance exercises of moderate level can be useful in the treatment of chronic lymphedema [32, 33].

Most importantly, there are growing body of evidence that regular moderate aerobic physical activity reduce the risk of relapse and significantly prolongs overall survival of breast cancer survivors (BCS) [34, 35]. This paper discusses the importance of primarily aerobic physical activity in terms of reducing the risk of relapse and impact on survival of these patients, and possible mechanisms underlying these effects, as well.

Definitions

Physical activity. It represents any movement of the body as a result of activity of skeletal muscles which substantially increases energy expenditure above basal levels (at least, 50% of maximum capacity for work or exercise) [36]. There are two main types of physical activities – aerobic, which increase aerobic capacity (walking, jogging, cycling, swimming, hiking) and non-aerobic, which increase muscle

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strength, elasticity and endurance, but not the aerobic capacity (exercise with resistance, stretching, yoga). In recent years, besides sports and recreational activities, usually performed in leisure time, physical activities also includes occupational and household tasks [37, 38].

Exercise – It is planned, ordered, purposeful and repetitive physical activity, performed with the aim of preserving or improving physical fitness [39].

Physical fitness – It is defined as a set of properties that people have or achieve by performing physical activity [40]. There are the four general categories of measurement of physical activity: calorimetry, physiological markers, motion detectors and questionnaires. The last ones are the most popular and practical approaches for large groups of individuals. A compendium of physical activities has been developed to provide researchers and practitioners with an estimation of the energy cost for a wide variety of human physical activities [37, 41].

Metabolic equivalent (MET) – MET is a unit used to express metabolic cost (oxygen consumption) of physical activity. One MET is the metabolic oxygen consumption in peace (at baseline) and it is equal to 3.5 mL O_2/kg/min. The metabolic cost of an activity expressed in MET indicates how many times is oxygen consumption higher during these activities in relation to consumption in peace [37].

Aerobic capacity – It is the ability of the organism to create the energy required for execution of any work with an aerobic process. It is measured by the amount of oxygen consumed during the performance of such work.

Maximal aerobic uptake (VO_2max) – VO_2max is the amount of oxygen consumed during the work of maximal intensity. As a measure of aerobic capacity, it was established as an international standard for physical fitness. It depends on gender, age and genetic heritage, but also on the level of aerobic physical activity [39, 42]. Today VO_2max is accepted as a predictor of mortality from cardiovascular disease and mortality of all causes of death, both in healthy populations and patients with different chronic diseases (diabetes, hypertension, hypercholesterolemia, chronic obstructive pulmonary disease), and smokers and obese persons, as well [43–45].

Body mass index (BMI) – BMI is the ratio between body weight and body height squared and expressed in kg/m^2. The normal BMI ranges between 18.5 and 25 kg/m^2. This is one of the widely accepted methods of obesity assessment [37].

Cancer survivor – It is any individual that has been diagnosed with cancer, from the time of diagnosis and for the balance of life [46]. This paper deals with women who have completed initial treatment for BC and have no apparent evidence of active disease.

Physical activity and body mass in women treated for breast cancer

Reduction of physical activity and increase of the body fat and body weight in breast cancer survivors (BCS) are two important risk factors for health. They are important not only for relapse of disease, but also for the morbidity from other chronic diseases such as arteriosclerosis, heart disease and diabetes. Although the risk of death due to primary disease remains elevated even 20 years after the diagnosis of breast cancer, the number of patients who die from other causes, such as cardiovascular disease is increasing [47].

According to the report of the International Agency for Research Cancer (IARC), about 25% of malignant diseases are the result of excessive weight and lack of physical activity [48]. Therefore, physical activity of these patients is of enormous importance. Yet, most patients diagnosed with malignant disease significantly reduce the extent and intensity of physical activity after the diagnosis compared to the period before the diagnosis.

Women with breast cancer are not an exception [49]. Taking into account antineoplastic therapy induced adverse events such as pain, nausea and fatigue, this trend is expected, although research shows that physical activity can help overcome these problems [8, 9, 50, 51].

In the Health, Eating, Activity, and Lifestyle (HEAL) multicenter study, conducted from July 1996 to March 1999, 1,185 women were enrolled 4–12 months after diagnosis of breast cancer. Among other objectives, the change of physical activity level of BCS after and before BC diagnosis was investigated and its impact on prognosis of the disease, as well. Data on physical activity were collected through a Modifiable Activity Questionnaire [52], and the average level of activity expressed in hours on a weekly basis. Authors analyzed 29 different activities (recreational and home activities) in addition to the time spent without the activity (reading, watching television, naps). Data were collected for two time periods – the period of 1 year before diagnosis and during the last month before the time of filling out questionnaires. A total of 812 women completed the study. It was shown that 52% of women with carcinoma *in situ*, 58% of women with locally and 62% with loco-regionally advanced disease reduced their overall level of physical activity. On average, an overall level of physical activity was decreased by 11%, corresponding to 2 hours *per* week, compared to the period before diagnosis. There was a significantly positive correlation between the type and intensity of physical activity and stage of the disease. Women with carcinoma *in situ* significantly reduced participation in sports / recreational and strenuous physical activities (≥ 6 MET), while women with locally or regionally advanced BC reduced the level of moderate activity (3–6 MET). The type of therapy is another factor that influenced the level of activity reduction. Women who had only surgery or radiotherapy were more active than those who received chemotherapy or combination therapy. It was also observed that the degree of obesity, expressed in BMI, is a good predictive factor for the level of physical activity. Overweight women had much more reduced activity (41.4%) than those whose BMI values were within normal limits (24.1%) or slightly higher than that (35.7%) [53].

Unfortunately, this trend of reducing physical activity continued in a follow-up period after initial treatment. Two years after the first interviews (within the third year of follow-up period) 806 women treated for BC billed out the

same questionnaire as in the previous study. The data showed that among these respondents, physical activity reduced and body weight and BMI increased in the last 3 years. In addition, women who had higher BMI had a lower total score of physical activity at all levels (moderate 28%, intensive 64%, and sports 49%) than women with normal BMI. Yet, during the first year after diagnosis, in the group with BMI < 30 kg/m^2 some increase in physical activity for the moderate and intensive activity was registered. It is interesting that women diagnosed with higher stage of disease (stage II) consumed 15% more time on physical activity than women with *in situ* disease. Among sports and recreational activities, walking was the most common and most popular activity. According to the U.S. Department of Health and Human Services recommendations (Surgeon General's physical activity recommendation) [39] at least 150 minutes of moderate to intensive activity (more than 3 MET) per week is necessary to maintain health. When observing all activities (total activity), this criterion was met by 73% of women. However, when household activities are excluded from the total score, only 32% of women in this study met the criteria of satisfactory level of activity [54].

It has long been observed that BCS often have a problem with weight gain, especially if they receive adjuvant systemic therapy [55]. In a large study, which included 1,116 women with operable breast cancer in stage I-IIIA disease during a 4-year follow-up period, it was shown that 60% of the patients increased body weight compared to the period before the disease diagnosis, 26% had reduction and 14% had no change. Factors that were positively and independently associated with obesity were: the time elapsed from diagnosis, adjuvant chemotherapy, African-American ethnic background, current energy intake and postmenopausal status at the time of study entry. Factors that were negatively and independently associated with obesity were: BMI before diagnosis, age at diagnosis, the level of education and current level of physical activity [56].

Physical activity and risk of relapse of disease and death in breast cancer survivors

One of the first longitudinal studies that confirmed the importance of physical activity in reducing the risk of death which was dose dependent was published in 2005. The study followed women diagnosed with BC during the period 1984–1998. By June, 2002, 2,987 women in stage I-III disease were followed. According to the level of physical activity expressed in MET-hours *per* week, subjects were divided into categories less than 3, 3–8, 9–15 and more than 15 MET-hours per week, while the group with the lowest level of activity was taken as a reference. The median follow-up period was 8 years. It was shown that the risk of death was significantly higher in the group of women not physically active enough (less than 9 MET-hours per week). This relationship was particularly pronounced among women with hormone-sensitive tumors: active women (more than 9 MET-hours *per* week) had a 50% lower risk of death than less active ones. The lowest risk of relapse and death was noticed in the group who had moderate levels of physical activity (9–15 MET-hours *per* week): the 5-year survival for this group was 97%, while for the group which was active less than 3 MET-hours *per* week, it was 93%. Moreover, with the increase in physical activity to some extent, the risk of death (specific for BC and other causes, as well) decreased, regardless of BMI [34].

Somewhat unexpected, the results of this study related to the fact that the risk of fatal outcome in a group of very intensive physical activity (more than 15 MET-hours *per* week) increased again. The authors associate this information with the impact of the disease stage at the time of diagnosis in women's behavior change. It was shown that women, if the diagnosis is worse, are prone to intensive lifestyle changes. In the group with intensive exercise were more women in stage II of the disease, while the group of moderate trainees comprised more women the stage I disease.

These observations are compliant with the results of the Health, Eating, Activity and Lifestyle (HEAL) study, which compiled data on physical activity 3 years after the BC diagnosis. Specifically, it showed that women with a higher stage of the disease at the time of diagnosis, had 15% more physical activity than those in whom ductal carcinoma (DCIS) *in situ* was diagnosed, although this difference was more in the domain of household activities [54]. In the following study, it was confirmed that the level of physical activity after BC diagnosis is more important from the standpoint of survival than the activity before the diagnosis. Women who increased their activity after BC diagnosis by 45% reduced the risk of death [57].

The latest meta-analysis on the effect of physical activity before and after the diagnosis of BC upon the survival and relapse of the disease, included the six major studies, with a total of 12,108 patients enrolled [58]. To enable the comparison of the results, the authors divided the level of physical activity in four categories: low, moderate, moderate to high and very high. The women from the low activity level group (less than 3 MET-hours per week*) were the reference group, used as basis for calculation of the results and the reference level for the comparison of the effects of physical activity on the risk of relapse and death. The results of three studies that examined the effects of primarily recreational activities before BC diagnosis on the risk of relapse and death show that physical activity before the diagnosis reduces the risk of death from all causes by 18%, but has no effect on mortality from BC [57, 59, 60]. However, women physically active after the BC diagnosis, compared with physically inactive ones, have 34% lower risk of death from breast cancer, and 41% lower risk of death from all causes. This is shown for all levels of physical activity. Also, moderate and moderate to high levels of physical activity after BC diagnosis reduce the risk of relapse by 24%, but this does not apply to a very high level of activity [58].

Two studies show that obese women (BMI > 25 kg/m^2) with more physical activity after BC diagnosis have a lower risk of death from breast cancer compared to less physically active women [57, 61], but this effect was not registered in

* Moderately fast walk (3–5 km/h) for 1 hour corresponds to the activities of 3 MET-hours.

women with normal body weight. On the other hand, more physically active women have a lower risk of death from all causes compared to the less active group, regardless of BMI [57, 62].

Possible mechanisms of influence of physical activity on disease relapse and survival

The mechanisms of physical activity reducing the risk of disease relapse and prolonging survival in BCS are as intriguing as the mechanism of its influence on primary prevention, and, probably, they are similar. The two most frequently mentioned mechanisms are: the impact on steroid hormones (especially on estrogen) [35, 63] and the impact on insulin function [35]. Both of these mechanisms are largely associated with the impact of physical activity on energy balance and body weight.

The importance of energy balance (the ratio between energy intake and expenditure) has long been observed from the standpoint of carcinogenesis and survival [64, 65]. For over 20 years it is well-known that women with increased body weight are exposed to two times higher risk of relapse within 5 years from diagnosis and 60% higher risk of death in the next 10 years, compared to women with normal weight (BMI < 25 kg/m^2) [66]. Central body fat distribution is associated not only with cardiovascular disease but also with some cancers [67–69]. Obesity is associated with the altered metabolism of estrogen [70], and facilitated conversion of androstenedione to estrogen [71, 72], as well as with the increase of insulin resistance, hyperinsulinemia and insulin-like growth factors [73]. According to current knowledge, it appears that exercise changes the pattern of accumulation of adipose tissue, leading to a reduction of abdominal fat [74]. This is especially important for postmenopausal women. After completion of reproductive age, the processes that lead to a reduction in muscle mass and increase in body weight occur more frequently, followed by the change of the distribution of body fat [75].

Sex steroid hormones have powerful mitogen and proliferative effects on breast tissue. Increased levels of circulating estrogen can stimulate the growth of breast cancer [76]. Possible mechanisms for estrogen induced breast cancer include increased breast epithelial cell proliferation, the metabolism of estrogen to genotoxic metabolites, such as DNA-adducts, and the silencing of tumor suppressor genes (TSGs) that have been implicated in breast carcinogenesis by inducing gene promoter hypermethylation, which is potentially reversible [63]. In contrast, lower levels of estrogen and progesterone in the circulation are associated with the reduction of breast epithelial cell proliferation [63, 77]. Because adipose tissue contains a large amount of aromatase, an enzyme that participates in the conversion of androgens into estrogens [72], obese BCS have increased risk of relapse of disease and death [78]. Physical activity can influence the reduction of fatty tissue, thus reducing the capacity of conversion of androgens to estrogens and lowering the levels of estrogen in the circulation [79]. Increased insulin resistance and increased amounts of insulin and insulin-like growth factor (IGF), and a decrease in insulin-like growth factor binding globulin (IGFBG) concentrations are often recorded in obese patients. Anabolic activity of insulin and IGF promote tumor growth by stimulation of cell growth and inhibition of apoptosis *via* the insulin receptor in breast tissue [80]. In addition, these two factors stimulate steroid hormones synthesis in the ovaries and inhibit sex hormone binding globulin (SHBG) production in the liver, which further emphasizes the process of carcinogenesis [81–83].

Acute physical effort improves insulin sensitivity and increases the uptake of glucose from plasma, mainly on the account of the activities of muscle tissue, but this reaction is lost within a few days [84]. However, regular physical activity of moderate or higher intensity maintains insulin sensitivity [84, 85] and protects against the development of insulin-independent diabetes [86]. It is assumed that this is a consequence of reduction in abdominal fat and increase of muscle mass, improved glucose transport in muscles [87] and/or reduced synthesis of fatty acids [84, 85]. In addition, the reduction of plasma insulin levels as a result of physical activity, leads to the production of a larger amount of SHBG which reduces the bioavailability of steroids in tissues, thus reducing the risk of disease relapse [88, 89].

A group of authors from Yale Medical School compared the level of free insulin, IGF-I and IGFBP-3 between the two groups of postmenopausal BCS. The first group had moderate level of regular aerobic activity of their choice for 30 minutes daily 5 times a week during a period of 6 months. The control group did not have specially organized activities, but continued with their normal daily activities. At the end of this period there was a significant difference in the level of IGF-I and IGFBP-3 between the groups. The level of free insulin was lower by 20.7% in the study group compared to the control group, but this difference was not statistically significant. The authors concluded that regular moderate aerobic activity, which was well tolerated by BCS, lowered the levels of IGF-I and IGFBP-3, whose activity was associated with disease relapse [90].

Physical activity – How much?

Previous researches show clearly a negative correlation between physical activity after diagnosis of breast cancer and the risk of disease relapse and death [91]. This correlation is dose- dependent [91] and it is more pronounced for postmenopausal women [62]. Apparently, this ratio is fairly stable, regardless of the level of dietary intake, body mass index, race, tumor stage and histological sub-type [92, 93].

A round table was organized in June, 2009 by the American College of Sports Medicine (ACSM), which brought together eminent experts in the field of rehabilitation of cancer patients, with the aim to search for the evidence of the impact of exercise on various aspects of life of cancer patients and survivors [91]. One of the aims was to provide guidance for treatment of such patients. There was consensus that the recommendations for the level of aerobic physical activity that exist for other chronic conditions are fully applicable to cancer survivors: 150 minutes of moderate to intensive or 75 minutes of very intensive aerobic activity *per* week (or

appropriate combination) [94–96]. In addition, it is recommended to perform exercises that involve large muscle groups, 2 to 3 times a week to strengthen muscles [94, 96]. Flexibility exercises are recommended before any of these two groups of exercises [97]. Of course, the level of physical activity must be adapted to the current state of the patient. According to the latest report of U.S. Department of Health and Human Services (U.S. DHHS) Physical Activity Guidelines for Americans [96], any exercise is better than nothing, and those who are unable to reach the recommended levels of physical activity should still be encouraged to be active as much as they can.

What kind of activities will be selected depends on individual choice, the current state of the body and overall ability of the patient. Walking is still the most popular form of aerobic training, which is entirely satisfactory if performed with the appropriate intensity. According to data from a sample of 2,987 women, intensive, fast walking had a similar effect as other intensive activities such as jogging, tennis or swimming [34].

Conclusion

Physical activity in BCS is particularly useful, not only because it improves physical and psychological health of survivors, but also because it reduces the risk of disease relapse and prolongs survival.

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Received on December 30, 2011.
Revised on September 20, 2012.
Accepted on November 1, 2012.

C A S E R E P O R T S

UDC: 617.576/.578::616.13/.14-007-056.7
DOI: 10.2298/VSP1401073K

Surgical treatment of hand vascular anomalies – a case report

Hirurško lečenje vaskularnih anomalija šake

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Abstract

Background. Vascular anomalies in the hand do not occur frequently. Their presence in the metacarpal region can cause different functional disorders. The extent of such disorders directly depends on the localization and size of vascular anomalies, duration and the nature of the anomaly growth as well as on eventual secondary complications such as ulceration or bleeding. The aim of this case report was to show the specifics in the clinical picture, pathogenesis and evolution of such anomalies, applied diagnostic procedures (radiography, ultrasound, magnetic resonance, electromyography) and surgical treatment as well as postoperative functional results. **Case report.** In the Clinic for Plastic Surgery and Burns, Military Medical Academy Belgrade, two patients were treated surgically for vascular anomalies of the middle palmar region of the hand. The first patient, a 36-year-old male, a former active sportsman (professional handball player) was treated for acute increase in the vascular anomaly of hand in the metacarpal region and proximal phalange of III and IV fingers of his right hand. The anomaly was detected 6 months prior to his hospitalization while two weeks before the hospitalization there was a sudden growth and increase in the change. The second case, a 15-year-old male patient actively pursuing a career in professional basketball was treated for a tumor localized in the metacarpal zone of his left hand. According to the information provided by his parents, the anomaly had been present since his birth. Initially, the anomaly manifested itself as a discoloration of the skin with a marked capillary drawing, gradually increasing throughout the last five years to the present dimension. The growth of the malformation was noticed to coincide in both patients with more active pursuit of their professional sports career. **Conclusion.** The clinical picture of hand vascular anomalies is dominated by the symptoms of compression of neurovascular structures (paresthesia, intense hand pain, swollen fingers). If it is a chronic progressive process, signs of ischemic intrinsic muscle fibrosis with corresponding functional deficit, as well as tissue defect (usura) of bone and joint structures represent the basic pathological findings. Acute increase is accompanied by compartment syndrome symptoms and ischemic fibrosis of intrinsic hand musculature and development of irreversible dysfunction of the hand. In the presented cases rapid response implies accurate diagnostic methods followed by surgical extirpation in order to treat compartment syndrome.

Key words:
vascular malformations; hand; diagnostic techniques and procedures; reconstructive surgical procedures; recovery of function.

Apstrakt

Uvod. Vaskularne anomalije u predelu šake ne javljaju se često. Njihovo prisustvo u zoni metakarpusa dovodi do različitih funkcionalnih smetnji šake. Obim tih smetnji direktno zavisi od lokalizacije i veličine vaskularne anomalije, trajanja i prirode rasta promene, kao i od eventualnih sekundarnih komplikacija kao što su ulceracija i krvarenja. Cilj ovog rada bio je da se prikažu specifičnosti kliničke slike, patogeneze i ocene tih promena sprovedene dijagnostičkim postupcima (radiografija, ultrazvuk, angiografija, magnetna rezonanca, elektromiografija) i načina hirurškog lečenja, kao i postoperativni funkcionalni rezultati. **Prikaz bolesnika.** U Klinici za plastičnu hirurgiju i opekotine Vojnomedicinske akademije u Beogradu hirurški su tretirana dva bolesnika sa vaskularnim anomalijama srednjedlanskog regiona šake. Prvi bolesnik, star 36 godina, muškog pola, bivši aktivni sportista (profesionalni rukometaš) lečen je zbog akutnog rasta vaskularne anomalije šake u metakarpalnoj regiji i proksimalnim falangama III i IV prsta desne šake sa palmarne strane. Vaskularna anomalija dijagnostikovana je šest meseci pre hospitalizacije, a nagli rast promene počeo je dve nedelje pre hospitalizacije. Drugi bolesnik, star 15 godina, muškog pola, aktivni profesionalni košarkaš, lečen je zbog tumefakcije lokalizovane u metakarpalnoj regiji leve šake sa palmarne strane. Heteroanamnestički podaci uzeti od roditelja ukazali su na to da je promena bila prisutna na rođenju bolesnika i da se manifestovala kao diskoloracija kože u predelu

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dlana sa kapilarnom šarom, koja je postepeno rasla, a naročito u poslednjih pet godina do aktuelne veličine. Rast opisanih malformacija kod oba bolesnika poklapao se sa povećanjem fizičke aktivnosti tokom njihovih sportskih karijera. **Zaključak.** U kliničkoj slici vaskularnih anomalija šake dominiraju simptomi kompresije na neurovaskularne strukture, od parestezija pa do intenzivnih bolova šake i otoka prstiju. Ukoliko se radi o hroničnom progredirajućem procesu, znaci ishemične fibroze intrinzičke muskulature sa odgovarajućim funkcionalnim deficitom, kao i uzure na koštanozglobnim strukturama, predstavljaju osnovni patološki nalaz. U slučaju akutnog rasta prisutni su simptomi kompartment sindroma sa pretećom ishemičnom fibrozom intrinzičke muskulature dlana i nastankom ireverzibilne disfunkcije šake. U tim, akutnim slučajevima brzo reagovanje podrazumeva precizne dijagnostičke metode, a nakon toga hirurišku ekstirpaciju u cilju lečenja kompartment sindroma.

Ključne reči:
krvni sudovi, malformacije; šaka; dijagnostičke tehnike i procedure; hirurgija, rekonstruktivna, procedure; oporavak.

Introduction

The majority of vascular anomalies develops within the first 2 years of life. Some of them are visible at birth with clear clinical signs which vary from skin changes that can include any discoloration, under-skin texture and consistency changes and may develop into hemangioma (growing congenital hemangioma), and the malformations which can appear after the birth on the not changed skin region [1, 2].

About 80% of vascular anomalies develop as a solitary tumor while there are about 20% of multiple proliferation cases. They are more common in female gender; this ratio ranging between 3–5 : 1.

The incidence is somewhat higher in the white race [2]. About 60% of vascular anomalies tend to appear in the cervical-facial region and this "tendency to expose" can cause a significant psychological stress both to parents and children [3].

In 1982 Mulliken and Glowacki described a clinically relevant classification of vascular anomalies, hemangiomas and vascular malformations, based on biological, pathohistological and clinical criteria [1].

Hemangioma is a benign tumor of endothelial cells, the cells that line blood vessels. Beside endothelial hyperplasia, histopathological findings show new vascular space formations composed of blood-filled "lakes" and channels as well as blood vessels. They have biphasic growth behavior: proliferative phase, which is, after achieving proliferative stage, followed by a slow involution, and in certain cases may end in total regression of the lesions when only regular observations are needed [4].

Vascular malformations comprise another category of vascular anomalies considered to be congenital errors in the process of vascular morphogenesis. Histopathological findings do not show elements of endothelial hyperplasia and, depending on chemodinamic characteristics, vascular malformations are subdivided into "low-flow" vascular anomalies (capillary malformation, venous malformation, lymphatic malformation, or combined malformation) and "high-flow" vascular anomalies (arteriovenous malformations). They both have a common characteristic: do not show any biphasic growth behavior, i.e. there are not proliferation or involution phases [1, 4, 5].

For arteriovenous hemangioma (malformations) plastic surgeons treatment is rarely needed, as long as its form, size, localization, a nature of growth and regression do not cause any functional and esthetic troubles. Only regular pediatric observations of children and parental education are needed [4].

Vascular anomalies (hemangiomas – arteriovenous malformations) rarely occur in the hand area. The most common site of the lesion is the palm of the hand [4]. Their presence in the metacarpal zone can cause functional disorder, dependent on their size and growth. A compression on neurovascular structures can cause paresthesia, burning or prickling of the hand, inclusive intense pain and swollen fingers.

If it is a progressive chronic process, signs of ischemic fibrosis of the intrinsic musculature with corresponding functional deficit as well as tissue defect of bone and joint structures represent the basic pathological findings [4, 5].

Case report

The first patient, a 36-year-old male patient was treated in our Clinic for acute increase of vascular anomaly of the hand in the metacarpal region and proximal phalange of III and IV finger of his right hand. Six months before his hospitalization, the former active sportsman (professional handball player) detected a change of 1 cm in diameter in the palm region of his right hand, which gradually increased.

Two weeks prior to hospitalization there occurred prompt growth and increase in vascular anomaly with significant symptoms of compression of neurovascular structures of the right hand palm followed by intense pain. On the day of admission to the hospital, a tumefact of 6 × 4.5 cm in diameter at the base and 1.5 cm high was detected on the palm of the right hand with lobular surface, dark blue color, without evident pulsations. The skin over the malformations was tense, hard and sporadically thinner. The hand was in the antalgic position and completely painful, finger flexion and extension limited, finger abduction and adduction were not possible (Figure 1).

Fig. 1 – The hand of the first patient preoperatively.

The preoperative clinical examinations and ultrasound findings showed on the right palm under the skin showed a soft tissue fluid formation detected with irregular canalicular form, filled with dense fluid (Figure 2).

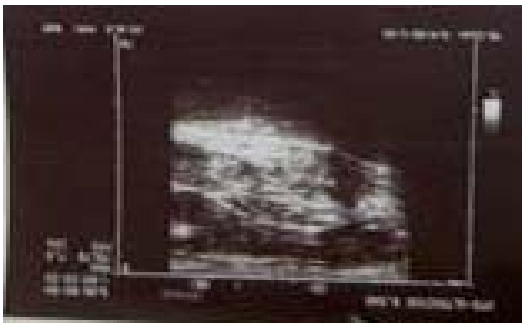


Fig. 2 – Preoperative ultrasound finding in the first patient.

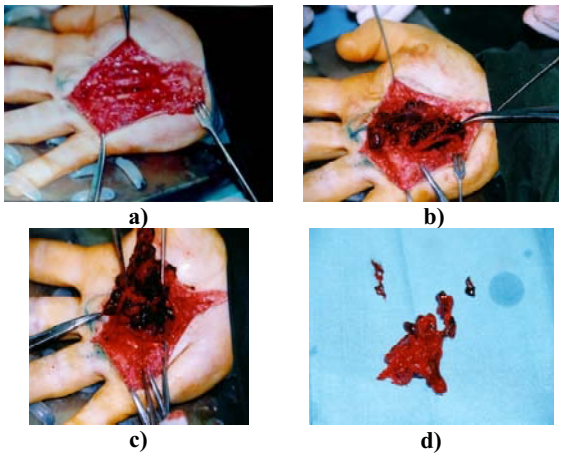
The percutaneous hemangiography findings on the palmar side of the hand showed irregular holes of angioma with superficial venous drainage network (Figure 3).

Fig. 3 – Percutaneous hemangiography finding in the first patient.

The malformation was extirpated in the "blood flow stasis". The zig-zag incision on the palmar skin brought a hemangioma just under it. Neurovascular elements and soft tissue structures of the middle palm region were identified under the tumor. In the radial part, a tumor segment was located under the muscle, while the rest of tumor tissue spreaded in the direction of the proximal parts of III and IV finger. Tumor tissue resection was done while neurovascular structures were preserved. Also, a partial usura in III metacarpal bone was detected. After partial release of the bandage, hemostasis was verified, aspirative drainage performed and the surgical wound closed with individual stiches (Figures 4 a–d).

The resected tumor was histopathologically proved as arteriovenous (AV) malformation – *haemangioma cavernosum*.

The rapid recovery of hand function was achieved with the sensitivity of all the fingers fully regained and the intrinsic musculature function completely restored (Figure 5) . The patient was referred to intensive physical therapy in the competent center for physical therapy and rehabilitation (Figure 6).



a) b) c) d)

Fig. 4 – Intraoperative finding of the surgical procedure and extirpation of hemangioma in the first patient.

Fig. 5 – The hand of the first patient postoperatively.

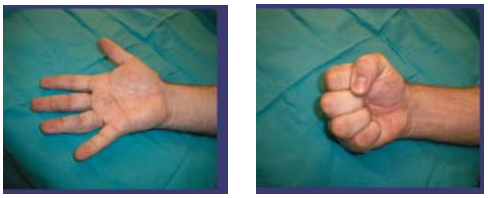


Fig. 6 – Functional result in the first patient 6 months after the operation.

The second case, a 15-year old male patient was treated in our Clinic for tumor localized in the metacarpal zone of his left hand. According to the information provided by the patient's parents the anomaly was present at birth. It was initially manifested as discoloration of the skin with marked capillary drawing, which gradually increased over the last five years in size to reach the actual dimension.

The change in growth coincided with the patient's more active pursuit of his professional sports career (basketball). He reported subjective discomphort as hand pain during weight training intensifying recently as well as discomfort during ball training exercises.

On the day of admission to the hospital, an oval form tumefact of 4 × 2 cm in diameter and 1 cm protruding out of the skin plane was detected on the middle region of the left hand palm, of light blue color, without clear limits, soft consistency and with palpable pulsations (Figure 7).

X-ray contrast angiography of the left hand showed intrapalmar hemangioma, which extended between metacarpal bones and partly along the first phalanx of the second finger.

Hemangioma itself was fed by *arcus palmaris profundus*, who gives two branches of the feeder. Hemangioma rapidly losed his stain in venous drainage through *arcus palmaris*, probably *profundus*, which was relatively *gracilis*. The venous phase of the initial part of *v. ulnaris* is showed (Figure 8).

Fig. 7 – The hand of the second patient preoperatively.

Fig. 8 – Preoperative angiography in the second patient.

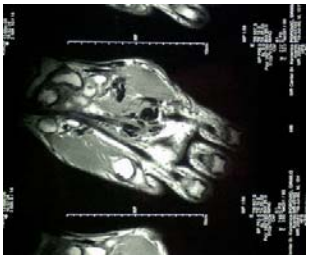
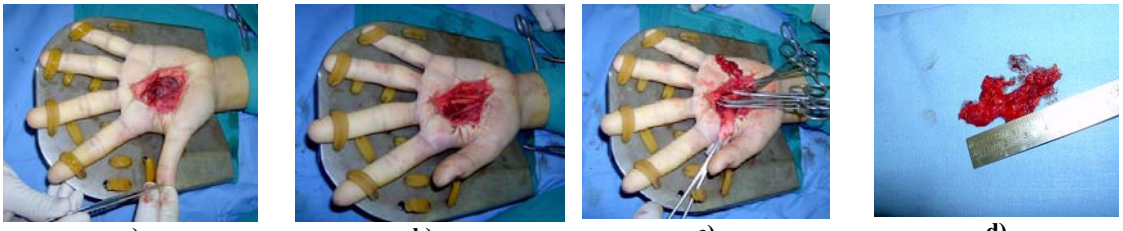


Fig. 9 – Preoperative magnetic resonance finding in the second patient.

Tumor resection was performed in the conditions of regional intravenous (RIV) anesthesia and blood flow stasis. The zig-zag incision was placed in the mid palm region. The tumor permeated the neurovascular and tendon elements, so after the tumor resection, all the elements were preserved.

The partial usure on the head of II metacorpal bone was detected. After the partial release of the bandage, the hemostasis was performed, using aspirative drainage, and the wound was closed with individual stiches in a single layer (Figures 10 a–d) .

Material for histopathological analysis represented vascular spaces, coated with flattened endothelial cells with the



a) b) c) d)

Fig. 10 – Intraoperative finding with extirpation of AV malformation in the second patient.

Magnetic resonance imaging of the left hand detected the apparent tangle of intertwined and dilated arterial blood vessels with its epicenter in the middle of the palm of approximate dimensions 6 × 4 × 3 cm. Due to the dense distribution of aberrant blood vessels it was difficult to define the superficial and deep palmar arch. It is certain that irrigation was done through the deep arcus since lumen expansion of the distal part of the deep branch of radial artery was detectable. A tangle of blood vessels spreaded between the tendons of deep and superficial finger flexors. Soft tissue components typical for hemangioma were not detected. Also, *usura* of the distal head and diaphysis of II metacarpal bone medial aspect was detected and therefore an intraaxial component of vascular malformations in the diaphysis of the same bone. On the place of the usura of the head medial aspect there were also criteria for intra-articular component of II metacarpophalangeal joint. Drainage was performed in the dorsal venous arch. The described vascular change of the palm of the left hand corresponded more to AV malformations than to the image of the classic hemangioma – possible AV type of hemangioma (Figure 9).

EMNG examinations registered practically regular findings with no signs of axonal affection and without malfunction of electrical stimulation of motor and sensory nerve fibers.

presence of vascular stents, surounded by. The received material did not show the elements of malignancy. The finding corresponded to vascular malformation.

A fast recovering of hand function was noticed, and after stitches removal, the patient was referred to intensive physical therapy (Figure 11).

Fig. 11 – The hand of the second patient postoperatively.

Discussion

Vascular anomalies in the mid-palmar region can cause functional disorders of various degrees, depending on the size, position and nature of growth.

The main symptoms were compressions of neurovascular structures in the form of paresthesia, numbness and a painful swelling of the fingers, with limited active mobility. The pressure on muscle, tendon and bone structure leads to fibrosis and atrophy of intrinsic hand musculature, limited active flexion and extension of fingers, as well as the appearance of usure on the bone joint structures.

Physical activities such as sport that requires full engagement of the hand, as was the case with the two patients treated at our Clinic, can accelerate the evolution of change and lead to a rapid growth phase. The possibility of complications due to injury of vascular anomaly in these cases is even larger. According to the reportes there are similar evidences about correlation of the expression and evolution of vascular anomalies and intensive phisical activites [6].

In case of acute rise the symptoms of compression of neurovascular bundle are expressed with signs of pain, paresthesia and loss of sensibility, as well as compression of intrinsic hand musculature, which significantly limits active and passive movements of the fingers – compartment syndrome.

Treatment of vascular anomalies includes various methods, which, considering the principle of *primum non nocere* provide a complete functional recovery, give acceptable esthetic results and prevent recurrence. Therapeutic modalities include observation, systematic and intralesional application of corticosteroids, use of interferon, laser and sclerotherapy and embolization, chemotherapy, cryotherapy, compression, surgical resection, and combined methods [7–11].

In cases of hand vascular anomalies, according to the anatomical and functional specificity of the hand, as well as possible complications that can cause disability, surgery is the choice of treatment. Other treatment methods, according to various data and theories in the literature (compression bandage, sclerotherapy, intralesional application of corticosteroids), may be applied only in a limited number of cases, as well as in a preparation for surgery which is a definitive method of treatment.

Conclusion

Depending on the size and rate of growth vascular anomalies in the mid-palmar region can lead to functional disorder of the hand of varying degree.

Complications that may result in disability can be avoided if a timely surgical intervention is applied after appropriate diagnostic procedures. A qualified physical therapy in the postoperative period enables a complete functional recovery of the affected hand, only if intervention was performed on time and if there was no irreversible damage of neurovascular, tendon and bone structures of the hand.

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Received on April 19, 2012.
Revised on September 26, 2012.
Accepted on October 10, 2012.

CASE REPORT

UDC: 616.136-007.64-08
DOI: 10.2298/VSP1401078S

Endovascular repair of ruptured abdominal aortic aneurysm

Endovaskularno lečenje rupture aneurizme abdominalne aorte

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Abstract

Introduction. Rupture of an abdominal aortic aneurysm (AAA) is a potentially lethal state. Only half of patients with ruptured AAA reach the hospital alive. The alternative for open reconstruction of this condition is endovascular repair (EVAR). We presented a successful endovascular reapir of ruptured AAA in a patient with a number of comorbidities. **Case report.** A 60-year-old man was admitted to our institution due to diffuse abdominal pain with flatulence and belching. Initial abdominal ultrasonography showed an AAA that was confirmed on multislice computed tomography scan angiography which revealed a large retroperitoneal haematoma. Because of patient's comorbidites (previous surgery of laryngeal carcinoma and one-third laryngeal stenosis, arterial hypertension and cardiomyopathy with left ventricle ejection fraction of 30%, stenosis of the right internal carotid artery of 80%) it was decided that endovascular repair of ruptured AAA in local anaesthesia and analgosedation would be treatment of choice. Endovascular grafting was achieved with aorto-bi-iliac bifurcated excluder endoprosthesis with complete exclusion of the aneurysmal sac, without further enlargment of haemathoma and no contrast leakage. The postoperative course of the patient was eventless, without complications. On recall examination 3 months after, the state of the patient was well. **Conclusion.** The alternative for open reconstruction of ruptured AAA in haemodynamically stable patients with suitable anatomy and comorbidities could be emergency EVAR in local anesthesia. This technique could provide greater chances for survival with lower intraoperative and postoperative morbidity and mortality, as shown in the presented patient.

Key words:
aortic aneurysm, abdominal; aortic rupture; comorbidity; diagnosis; vascular surgical procedures; blood vessel prosthesis; treatment outcome.

Apstrakt

Uvod. Ruptura aneurizme abdominalne aorte (AAA) predstavlja potencijalno smrtonosno stanje. Samo polovina bolesnika sa rupturom AAA stigne živa u bolnicu. Alternativa otvorenoj hirurškoj rekonstrukciji ovog stanja je endovaskularno lečenje. U radu je prikazano uspešno izvedeno endovaskularno lečenje rupturisane AAA kod bolesnika sa velikim brojem pratećih oboljenja. **Prikaz bolesnika.** Muškarac star 60 godina, primljen je u našu instituciju zbog difuznih bolova u trbuhu praćenih nadimanjem i podrigivanjem. Na ultrazvučnom pregledu abdomena viđena je AAA, što je potvrđeno i multislajsnom kompjuterizovanom angiografiji abdomena, koja je ukazala i na postojanje velikog retroperitonealnog hematoma. Zbog pratećih bolesti (prethodna operacija karcinoma larinksa i suženje larinksa na jednu trećinu, arterijska hipertenzija i kardiomiopatija sa ejekcionom frakcijom leve komore od 30%, stenoza desne unutrašnje karotidne arterije od 80%) odlučeno je da je lečenje izbora rupture AAA endovaskularno, u lokalnoj anesteziji i analgosedaciji. Endovaskularni grafting postignut je ugrađivanjem aortobiilijačne bifurkacione ekskluder endoproteze sa potpunom ekskluzijom aneurizmatske vreće, bez daljeg uvećanja hematoma i isticanja kontrasta. Postoperativni tok protekao je uredno, bez komplikacija. Na kontrolnom pregledu tri meseca kasnije, stanje bolesnika bilo je dobro. **Zaključak.** Endovaskularno lečenje rupturisane AAA može biti terapija izbora kod hemodinamski stabilnih bolesnika sa značajnim komorbiditetima ukoliko morfologija aneurizme to dozvaljava. Ova tehnika pruža veću šansu za preživljavanje, uz manji stepen intraoperativnog i postoperativnog morbiditeta i mortaliteta što potvrđuje i prikaz našeg bolesnika.

Ključne reči:
aorta, abdominalna, aneurizma; aorta, ruptura; komorbiditet; dijagnoza; hirurgija, vaskularna, procedure; krvni sud, proteza; lečenje, ishod.

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Introduction

Abdominal aortic aneurysm (AAA) is the pathological, over 50%, enlargement of the aorta. It could develop in both men and women, usually in the elderly [1]. The incidence and prevalence of both AAA and ruptured AAA continues to increase. In the United Kingdom this is the 8th commonest cause of death, responsible for 10,000 to 12,000 deaths *per* year [2, 3]. Progressive aneurysm enlargement can lead to rupture and massive intra-abdominal bleeding with fatal outcome, unless timely repair can be achieved. There were 6,800 deaths in the 2000 in England and Wales due to rupture of AAA [3]. Despite improvements in perioperative and postoperative management, the mortality rate remains high after conventional open surgical repair. The overall mortality rate from aneurysm rupture ranges between 65% and 85%[4]. The half of deaths from ruptured AAA not even reach the hospital and for those who survive the initial period the mortality rate in open emergency surgical repair ranges between 30% and 70% [4, 5]. As a newer minimally invasive technique, endovascular repair showed improvement in reduction of an early morbidity and mortality, as compared to conventional open surgery repair for elective treatment of AAA [6, 7]. As elective endovascular aneurysm repair (EVAR) provides a significant reduction in aneurysm-related mortality for 30 days (as well in 4 years follow-up), it may be speculated that this technique could offer an improvement in long-term survival for patients with ruptured AAA [8]. Emergency EVAR in the treatment of ruptured AAA has been used with success, proving that it is feasible in selected patients. However, it is not known yet will emergency EVAR provide significant improvement in early and late mortality for these patients, nor could it replace conventional open repair as the preferred treatment of this lethal condition.

Case report

A 60-year-old man was admitted to our institution due to diffuse abdominal pain with propagation to the sacroiliac region more to the left side, lasted for 2 days and accompained by flatulence and belching. Initial abdominal ultrasonography showed AAA with maximum diameter 70 mm with retroperitoneal haemathoma in the left paracolic space. As the patient's clinical state was haemodynamically stable, urgent computed tomography (CT) scan aortography was performed. CT showed infrarenal AAA with a maximum anteroposterior diameter of 72 mm. There were a large retroperitoneal haematoma extending into the left iliopsoas muscle and left paracolic gutter (Figure 1).

The patient omentioned the previous surgery of laryngeal carcinoma in another medical institution (2 years before), and showed recent otorhinolaryngological findings with the confirmed existence of one-third laryngeal stenosis. Also, the patient had arterial hypertension with irregular drugs intake, and cardiomyopathy with documented left ventricle ejection fraction of 30% confirmed by the cardiologist on admission to our institution. Visual disturbances (amaurosis fugax) verified a high grade stenosis of the right internal carotid artery (80%) with elevation of systolic blood

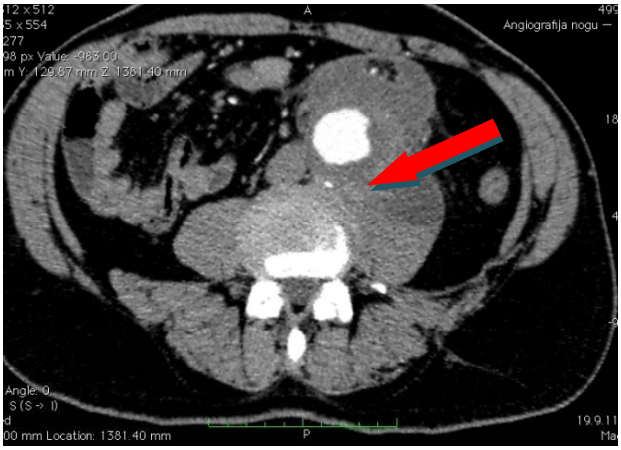

Fig. 1 – Infrarenal suprailiac aneurysm of the abdominal aorta with the maximal anteroposterior diameter of 72 mm and the large retroperitoneal haematoma extending into the left iliopsoas muscle and left paracolic gutter on multislice computed tomography scan angiography.
(Retroperitineal hematoma marked with a red arrow)

flow velocity and turbulence on colour doppler ultrasound in another medical institution. In view of high comorbidities the patient was considered unfit for open surgery and general anaesthesia. The appearance of CT (aneurysm's neck length 20 mm and favourable iliac arteries) suggested that the patient was suitable for endoluminal stent grafting. Under local anesthesia (20 mL of lidocaine-chloride in concetration of 2%) and analgosedation followed by continuous monitoring of vital function the emergency EVAR was performed. We selected an aorto-bi-iliac endovascular stent graft using a Siemens Siremobil® Iso-C Image Intensifier (Siemens AG, Erlangen, Germany). Access was gained *via* bilateral femoral approach. Table angiogram with a measuring catheter showed infrarenal AAA without extension into the common iliac arteries. An aorto-bi-iliac bifurcated excluder endoprosthesis (WL Gore, USA; size PXT 231416 and contralateral PXC 141400) was positioned and deployed infrarenally under fluoroscopy guidance using standard techniques.

Control angiography showed complete exclusion of aneurysmal sac, without further enlargment of haemathoma and no contrast leakage (Figures 2 and 3). During operation the patient received 5000 I.U. /mL of heparin-sodium solution and 1.5 g of cefuroxime intravenously. The procedure lasted for 70 min, without complications. The patient was transferred to the surgical intensive care unit where he was closely monitored for one day, with administering of two units of fresh blood supstitution therapy followed by standard therapy. On the 7th postoperative day an endarterectomy of the right internal carotid artery was performed in general anaesthesia and video-assisted tracheal intubation, without intraoperative and postoperative complications. Further state of the patient was eventless, without complication and he was discharged from the hospital after 12 days of the second operation. On recall examination 3 months after patient's discharge from the hospital, the state of the patient was well, with no complaints related to AAA.

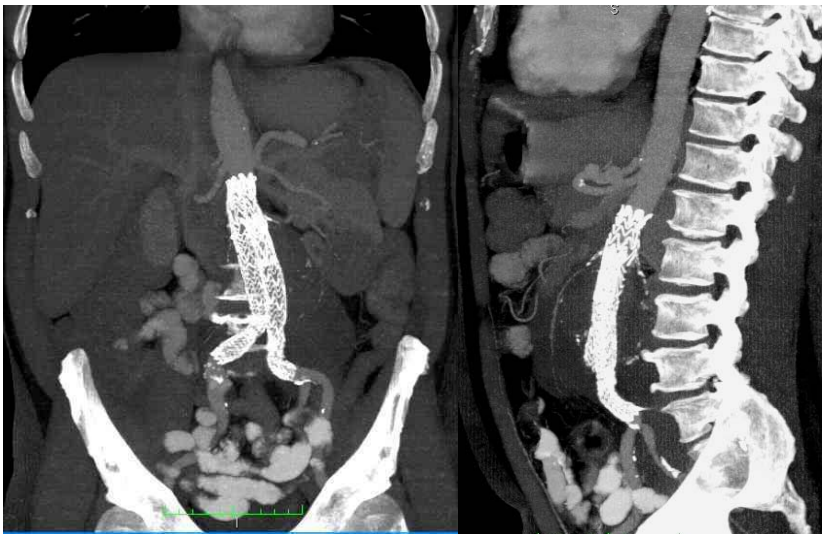

Fig. 2 – An aorto-bi-iliac bifurcated excluder endoprosthesis positioned and deployed infrarenally.

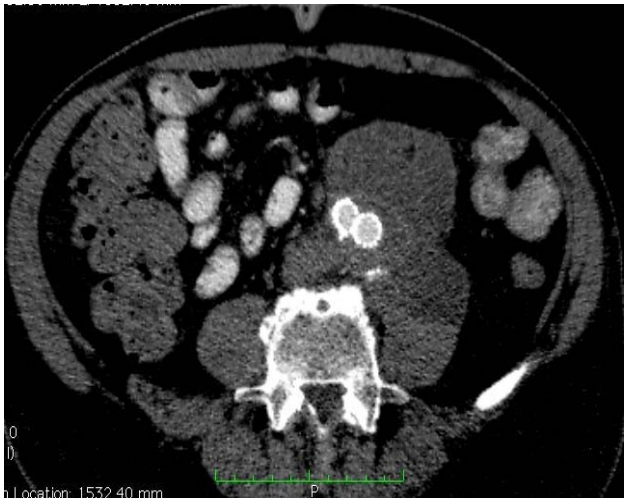

Fig. 3 – Complete exclusion of the aneurysmal sac, without enlargment of haemathoma and no contrast leakage on post deployment computed tomography scan angiography.

Discussion

Following rupture of AAA, only 38–64% of patients reach the hospital alive [9]. Rupture of AAA can result in haemodynamic collapse and death before hospital admission of patients. Operative treatment of ruptured AAA is associated with mortality rates between 25% and 60% [10]. In such patients with ruptured slowly blood leaking AAA, the clinical picture is ussualy presenting with abdominal pain, pulsating abdominal mass and hypotension. AAA rupture is more frequent in patients with chronic obstructive pulmonary disease, cardiac disease, stroke and aneurysm size greater than 5 cm [11]. The same factors in addition to malignancy, chronic renal failure, recent myocardial or cerebal infarction, dementia, coexistent severe acute pancreatitis and oral anticoagulant therapy make patients unfit for open surgery repair [10, 12].

Endovascular repair of AAA is minimally invasive procedure accompanied with the reduction in morbidity and mortality during and after the procedure, regarding the need for surgical exposure of the aorta and aortic cross clamping in open surgical reconstruction [13, 14]. Further, usage of endoprosthesis can considerably reduce cardiac strain during the procedure as compared to open surgery and hence is particularly advantageous in surgically high risk patients [15]. During endovascular repair of AAA, an endograft is positioned within the aorta by the transfemoral approach to exclude aneurysmal sac from circulation [13]. It was showed that covering by stent seal off AAA immediately after stent placement, followed with gradual obliteration of aneurysm sac by collagen reduce risk of further aneurysm expansion and rupture [16]. The data from the Registry of Endovascular Treatment of Abdominal Aortic Aneurysms (RETA) show that only 30–50% of aneurysms are suitable for endovascular repair [14]. The procedure requires accurate determination of aneurysm morphology with contrast enhanced MSCT angiography. In symptomatic and ruptured AAA, MSCT scan can rapidly assess the feasibility of endovascular treatment [14, 16]. It is provided in patients with haemodynamically stable condition, as in our case. Due to simplicity, reduction in haemodynamic manipulations, reduced requirement for both intravenous fluid administration and subsequent monitoring of patients, the need for stay in intensive care units and less of hospital beds, local anaesthesia combined with analgosedation is an acceptable technique for endovascular repair of aneurysms [15]. Also, this type of anaesthesia provides a particular advantage in patients with high risk for cardiovascular complications, since it does not change the haemodynamic situation in contrast to general anaesthesia [15, 17]. Comparing the usage of general, epidural and local anaesthesia for endovascular repair of aneurysms, Bettex et al. [15] successfully used local anaesthesia in 63 of 91 patients, including 4 patients with ruptured aneurysm. The poor cardiopulmonary reserve in the presented patient made him unsuitable for general anaesthesia. For now, it seems that endoluminal repair of ruptured AAA is only applicable in selected haemodynamically stable patients, unfit for open surgical

procedure [12, 13]. Since the report by Yusuf et al. [18] in 1994 showed the feasibility of EVAR in ruptured AAA, there have been others from specialised vascular units limited to selected cases and in haemodynamically stable patients. Those series included variety of aortic ruptures, such as aorto-caval, aorto-renal vein and enteric fistulas, and rupture of false aneurysms following open surgical repair [19–22]. In the last ten years various series and prospective studies showed that EVAR could offer another option for the patients with ruptured AAA [19–23]. The presented patient is the second reported case with ruptured AAA treated by EVAR in Serbia [24], but the first one successfully treated due to acute ruptured AAA.

The main disadvatage of EVAR is the high costs of stent-grafts and their delivery systems, plus the cost of any adjunctive procedures. Also, the need for lifelong follow-up imaging and long-term durability of graft material is yet to be proven [25]. Considering the fact that each patient requires the appropriate stent-graft, at least one day is needed for its procurement even in the most developed countries. The shortest period to purchase appropriate stent-graft in Serbia is two days [24]. Regarding the presented patient we had an appropriate stent graft which was intended for another patient. Although a recently published retrospective analysis of 651 patients who underwent EVAR and open repair of elective infrarenal AAA shows that the 30-day mortality rate after open repair is similar to that after EVAR in patients younger than 60 years [26], it is not known yet if emergency EVAR will lead to significant improvements in outcomes for these patients, nor could it replace conventional open repair as the preferred treatment for ruptured AAA. The Immediate Management of the Patient with Rupture (IMPROVE aneurysm trial open *versus* endovascular repair) will in the near future answer to the question if patients with ruptured AAA derive benefit from EVAR regarding 30-day mortality, 24 hours in-hospital and one year mortality, complications and morbidities, as weel as quality of life, costs and cost-effectivenes [27, 28].

Conclusion

The half of patients with ruptured AAA die before reaching the hospital. Intraoperative and postoperative mortality rate of open surgical repair is high especially in patients with comorbidities. The alternative for open reconstruction of ruptured AAA in haemodynamically stable patients with suitable anatomy and comorbidities could be emergency EVAR in local anaesthesia.

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Received on March 30, 2012.
Revised on December 5, 2012.
Accepted on December 10, 2012.

CASE REPORT

UDC: 612.017:616.36-02/-08
DOI: 10.2298/VSP1401083A

Primary biliary cirrhosis and hepatic sarcoidosis – A case report

Primarna bilijarna ciroza i sarkoidoza jetre

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Abstract

Introduction. Primary biliary cirrhosis (PBC) is an immune-mediated chronic progressive inflammatory liver disease leading to destruction of small interlobular bile ducts. Sarcoidosis is a chronic disorder of unknown etiology characterized by non-caseous granulomas. **Case report.** We reported a 69-year-old female patient with abdominal pain, malaise, vertigo, headaches, hands tremor and partial loss of hearing. Initial laboratory findings revealed elevated liver function tests and cholesterol with positive antimytochondrial and antinuclear antibodies. Liver biopsy revealed granuloma typical for PBC and granulomatous lesions typical for sarcoidosis. Elevated serum angiotensin-converting enzyme and granulomatous lesion on the brain magnetic resonance imaging (MRI) were detected and the patient was diagnosed with overlap of PBC and liver sarcoidosis and neurosarcoidosis. The patient was treated with ursodeoxicholic acid (UDCA) and prednisolone. Six months later the patient was symptom-free with laboratory findings within normal range. **Conclusion.** In PBC patients it is important to consider coexisting granulomatous liver diseases if elevated liver function tests persist despite UDCA therapy.

Key words:
liver cirrhosis, biliary; sarcoidosis; diagnosis, differential; histological techniques; treatment outcome.

Apstrakt

Uvod. Primarna bilijarna ciroza (PBC) je imunološka, hronična, progresivna bolest jetre koja dovodi do destrukcije malih, interlobularnih žučnih kanala. Sarkoidoza je hronična bolest nepoznate etiologije koju karakterišu nekazeozni granulomi. **Prikaz bolesnika.** Prikazana je bolesnica stara 69 godina, sa bolom u trbuhu, malaksalošću, vrtoglavicom, glavoboljom, tremorom i delimičnim gubitkom sluha. Laboratorijske analize ukazale su na povišene vrednosti enzima jetre, holesterola i pozitivna antimitohondrijalna i antinuklearna antitela. Biopsijom jetre dokazani su granulomi tipični za PBC i granulomske lezije karakteristične za sarkoidozu. Nalaz povišenih vrednosti angiotenzin-konvertujućeg enzima i granuloma na magnetnoj rezonanci endokranijuma doveli su do dijagnoze PBC i sarkoidoze jetre uz neurosarkoidozu. Bolesnica je lečena ursodeoksiholnom kiselinom (UDCA) i prednizolonom. Nakon šest meseci praćenja bolesnica je bila bez simptoma uz normalne vrednosti laboratorijskih analiza. **Zaključak.** Kod bolesnika sa PBC potrebno je razmotriti postojanje drugih granulomskih bolesti jetre ukoliko poremećaj hepatograma perzistira nakon uvođenja UDCA u terapiju.

Ključne reči:
jetra, bilijarna ciroza; sarkoidoza; dijagnoza, diferencijalna; histološke tehnike; lečenje, ishod.

Introduction

Primary biliary cirrhosis (PBC) [1, 2] is an immune-mediated progressive chronic liver disease. In course of the disease destruction of small interlobular bile ducts together with progressive cholestasis is observed. If PBC is not treated it progresses to liver fibrosis and cirrhosis. PBC affects middle-aged women. Biochemical markers in PBC patients reveal cholestasis together with positive finding of serum antimitochondrial autoantibodies (AMA) [3]. Histological examination after liver biopsy reveals damaged biliary epithelial cells and loss of small intrahepatic bile ducts. In the portal tracts $CD4^+$ and $CD8^+$ T cells, B cells, together with macrophages, eosinophils and natural killer cells are found [1, 4].

Sarcoidosis is chronic granulomatous disease of unknown etiology that can affect different and sometimes multiple organs. It is most commonly diagnosed in patients aged 25 to 40, more often in women [5]. Pathogenesis of the disease is not completely clarified but it is probable that in geneti-

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cally susceptible individual immunological response to yet unidentified antigen triggers of the disease exists. Loss of both regulatory and apoptotic mechanisms are also proposed as possible factors that play a role in the pathogenesis of this disease [6]. Inflammatory process leading to granuloma formation is predominantly T helper 1 (Th1) mediated and involves lymphocytes, macrophages and cytokines [7]. Underlying mechanism responsible for chronic course and fibrosis in sarcoidosis patients is unclear. The majority of sarcoidosis patients remain asymptomatic despite the fact that hepatic granulomas are found after liver biopsy in 50–65% of patients and in autopsy series in 70% of cases [8]. Liver function tests remain within normal range in the majority of cases and chronic intrahepatic cholestasis progressing to biliary cirrhosis is rare [8].

Since in many patients with PBC histological examinations of the liver reveal hepatic granulomas and taken into account similarities in clinical manifestation possible relation between PBC and sarcoidosis is presently under debate.

Nevertheless, evidence of overlap of these two diseases is extremely rare, with the majorty of published data related to patients with skin sarcoidosis and PBC [9–12].

Case report

A 69-year-old female patient was referred to the Clinic for Gastroenterology, Clinical Center of Serbia, from regional hospital where, based on elevated liver function tests and positive AMA, PBC was diagnosed and the patient was started on standard dose of ursodeoxycholic acid (UDCA). The patient was referred to our Clinic for further diagnosis and treatment since laboratory findings did not improve in the course of UDCA therapy and the patient was experiencing clinical symptoms. On admission the patient reported abdominal pain localized in right upper quadrant, exhaustion, occasional vertigo, headaches localized in the occipital region, tremor of the hands and unilateral partial loss of hearing. The patient's past medical history was not significant. Physical examination revealed xanthelasma on eyelids. Neurological examination revealed partial unilateral hearing loss and postural tremor of the hands. Initial laboratory findings revealed hemoglobin (Hgb) 100g/L (normal range 122–157 g/L), mean corpuscular volume (MCV) 74.6 (normal range 83–97.2 fL), hematocrit (Htc) 30% (normal range 35.6–47%), serum iron 4.2 µmol/L (normal range 7–28 µmol/L), total iron binding capacity (TIBC) 79.4 µmol/L (44.8–80.6 µmol/L), ferritin 3.1 µg/L normal range (21.8–274.7 µg/L, aspartate aminotransferase (AST) 48 U/L (normal range 0–37 U/L), alanine aminotranferase 67 U/L (normal range 0–41 U/L), cholesterol 8.25 mmol/L (normal range 0–5.2 mmol/L). Hepatitis A, B and C profiles were negative. Alpha1 antitrypsin and ceruloplasmin were within normal range. Immunology profile revealed positive AMA-M2, 1:640, and antinuclear antibodies (ANA) – Hep2 IgG: cytoplasm + mitochondrial type 1:320. Further investigation revealed all tested tumor markers including AFP, CEA, CA 19-9 and CA 125 were within normal range. Chest X-ray and abdominal ultrasound were normal. On the esophagogastroduodenoscopy multiple angiodysplasia in the duodenal mucosa were detected and treated with argon plasma coagulation. Colonoscopy and barium follow-through were normal. Video capsule endoscopy excluded the presence of other causes of anemia apart from previously diagnosed duodenal angiodysplasia. Percutaneous liver biopsy was performed and histological examination revealed granuloma typical for PBC (Figure 1) and unexpectedly also granulomatous lesions typical for sarcoidosis (Figure 2).

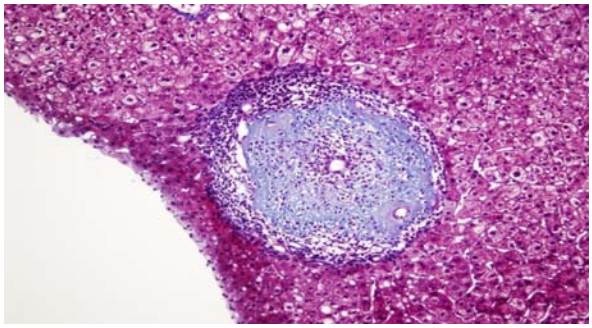

Fig. 1 – Granuloma typical for primary biliary cirrhosis (HE, × 200).

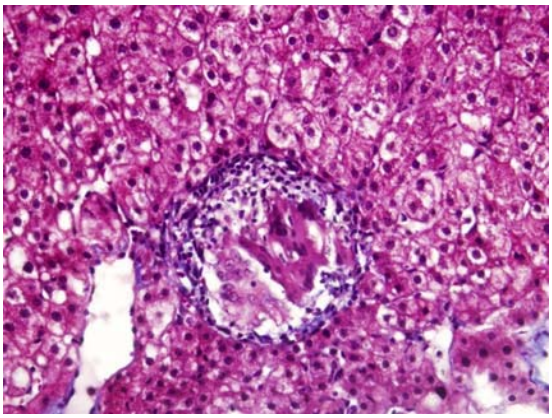

Fig. 2 – Granulomatous lesions typical for sarcoidosis (HE, × 400).

Since liver biopsy suggested overlap of PBC and sarcoidosis further diagnostic procedures were performed and revealed elevated serum angiotensin-converting enzyme (ACE) of 88 U/L (range 8–52 U/L), while chest computed tomography (CT) scan was normal thus excluding pulmonary sarcoidosis. Since the patient had neurological symptoms (headache, vertigo, unilateral partial loss of hearing and tremor of the hands) neurological workup was performed and it revealed ACE levels of 8 U/L in cerebrospinal fluid, while brain magnetic resonance imaging (MRI) revealed focal, nodular lesion localized in the right cavernous sinus near internal carotid artery and medial cerebral artery. This lesion had MRI characteristics of granulomatous lesion (Figure 3). In differential diagnosis aneurismatic dilation of blood vessel was considered but excluded after MRI brain angiography was normal. The diagnosis of neurosarcoidosis was established.

Fig. 3 – Brain magnetic resonance imaging (MRI) with focal, nodular lesion localized in the right cavernous sinus near internal carotid artery and medial cerebral artery. This lesion has MRI characteristics of granulomatous lesion.

After detailed diagnostic workup the patient was diagnosed with overlap of PBC and liver sarcoidosis and neurosarcoidosis. The patient was continued on UDCA and started on 20 mg of oral prednisolone. Six month later the patient was symptom-free and laboratory findings were within normal range.

Discussion

Gastrointestinal tract is rarely affected by sarcoidosis. Gastric sarcoidosis is diagnosed in 2.5%, and intestinal involvement in 3.4% of all sarcoidosis patients [13]. As opposed to these findings liver involvement is diagnosed and granuloma typical for sarcoidosis detected after liver biopsy in 50–79% of patients, while autopsy reveals granuloma in 67–70% of patients [8, 13].

Liver involvement is more frequent in African Americans than in Caucasians [14]. Symptomatic liver disease is rare despite frequent granuloma detection. Abdominal pain and itching are more frequent than jaundice. Fever is more frequently seen in patients with liver sarcoidosis than in those without liver involvement [15]. The responsible pathogenic mechanisms resulting in jaundice are intrahepatic cholestasis, but also hemolysis and hepatocellular dysfunction. Granulomatous hilar lymph nodes can also cause obstruction of extrahepatic bile ducts and result in jaundice [13].

Abnormal values of liver tests found in 35% of patients with sarcoidosis are not reliable predictors of clinical course and outcome of the liver disease [16]. Approximately one quarter of sarcoidosis patients have liver involvement with no pulmonary disease. Enlarged liver is diagnosed during physical examination in one out of five patients while abdominal CT scans show liver enlargement in more than 50% of patients [13]. Hepatic granuloma can be found on liver biopsy irrespective of the liver size thus biopsy should be performed in cases of suspected liver sarcoidosis even if the liver is not enlarged. Alkaline phosphatase is a more reliable marker of liver involvement than gamma glutamyl-transpeptidase.

Overlap syndrome or simultaneous presence of PBC and sarcoidosis has been previously reported, and the majority of cases were of skin and/or lung sarcoidosis [9, 10, 12], while the simultaneous presence of hepatic sarcoidosis and PBC [5, 11] is very rare. Namely Rajoriya et al. [5] analyzed data from 1,510 patients with sarcoidosis aiming to identify disease association of sarcoidosis with different immune mediated and chronic inflammatory disease. Out of 1,510 patients only in 3 PBC was diagnosed and these results suggested that there was no significant association of PBC with sarcoidosis implying that this overlap is rare [17]. To the best of our knowledge this is the first case of neurosarcoidosis described in PBC patient.

Histological similarities between PBC and sarcoidosis described more than 35 years ago tempted authors to speculate close relationship of the two diseases and to suggest possible common pathogenesis [18]. Karlish et al. [19] reported a female patient with cholestatic liver disease, enlarged hilar and paratracheal lymph nodes. The diagnosis of sarcoidosis was based on positive Kweim test, while positive AMA supported the diagnosis of PBC. A year later a case series was published demonstrating the simultaneous presence of PBC and liver sarcoidosis in 10% and PBC with lung sarcoidosis in another 10% of patients [20]. Stanley et al. [21], Rudzki et al. [22] and Fagan et al. [23] also reported small series of PBC patients with lung sarcoidosis, but none of these patients suffered from liver sarcoidosis. Hughes and McGavin [11] reported a female patient diagnosed with sarcoidosis affecting her skin, nasal mucosa, lungs, lacrymal and parotid glands. In that patient granulomatous hepatitis with immunological features of PBC was diagnosed together with myositis.

One of the most detailed reports published by Stanca et al. [17] deals with a patient with progressive cholestatic liver disease that lead to liver transplantation. In this particular patient the differential diagnosis between liver sarcoidosis and PBC was not resolved during 24 years (from 1980 till 2004). Since the patient was diagnosed with lung sarcoidosis liver granuloma were considered to be related to sarcoidosis until the positive findings of AMA that confirmed the diagnosis of PBC.

The number of reported sarcoidosis and PBC overlap cases is too small to support the etiological link between the two diseases. The diagnosis of PBC is in 90% of patients confirmed by positive AMAs test result since AMA are negative in liver sarcoidosis.

Use of immunoblotting and enzyme-linked immunosorbent assay (ELISA) to detect M2-specific AMAs, as well as adequately performed and analyzed liver biopsy will probably result in precise establishing of the incidence of overlap between PBC and liver sarcoidosis, resulting in prompt treatment that will result in improved quality of life and prognosis of these patients.

Conclusion

It is of great importance to consider coexisting sarcoidosis and other liver diseases in PBC patients especially if elevated liver function tests persist despite the therapy.

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Received on June 13, 2012.
Revised on July 3, 2012.
Accepted on August 23, 2012.

CASE REPORT

UDC: 616-001-053.2:[616.728.3-001+616.13/.14-001
DOI: 10.2298/VSP1401087C

Popliteal artery injury following traumatic knee joint dislocation in a 14-year-old boy: A case report and rewiev of the literature

Povreda poplitealne arterije nakon traumatske dislokacije zgloba kolena kod 14-godišnjeg dečaka: prikaz bolesnika i pregled literature

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Abstract

Introduction. Posterior knee joint dislocation associated with injury of the popliteal artery in children is an extremely rare condition. Rapid diagnosis and treatment are essential for limb salvage and function. **Case report.** We reported a 14-year-old boy who suffered traumatic displacement of the right knee and contusion of the popliteal artery during motorcycle accident. The diagnosis was confirmed using Doppler and duplex ultrasonography and digital substraction transfemoral arteriography. The urgent surgical procedure was performed using posterior approach to the popliteal artery. During the surgical exploration, rupture of the posterior cruciate ligament associated with thrombosed popliteal artery have been found. The damaged popliteal artery was resected and replaced with autologous saphenous vein graft. The last stage of the procedure was a transosseous femoral fixation of posterior circuate ligament. A 3-year-follow-up after the surgery demonstrated intact arterial perfusion and very good function of the knee with a minimal difference as compared with the contralateral knee. **Conclusion.** Combined orthopedic and vascular injuries are very rare in children. They require combined treatment.

Key words:
knee injuries; popliteal artery; blood vessels; wounds and injuries; child; vascular surgical procedures; transplants; recovery of function.

Apstrakt

Uvod. Zadnja dislokacija zgloba kolena praćena povredom poplitealne arterije kod dece veoma je retko stanje. Brza dijagnoza i lečenje ključni su za očuvanje kako samog ekstremiteta tako i njegove funkcije. **Prikaz bolesnika.** Prikazali smo 14-godišnjeg dečaka koji je pretrpeo iščašenje desnog kolena i kontuziju poplitealne arterije u saobraćajnoj nesreći na motociklu. Dijagnoza je potvrđena doplerom i ultrazvučnim dupleks pregledom i digitalnom suptrakcionom transfemoralnom arteriografijom. Urađena je hitna hirurška procedura zadnjim pristupom poplitealnoj arteriji. Tokom hirurške eksploracije pronađena je ruptura zadnjeg ukrštenog ligamenta udružena sa trombozom zatkolene arterije. Oštećena poplitealna arterija resecirana je i zamenjena autolognim safenskim venskim graftom. Poslednji korak hirurške procedure bio je transosealna femoralna fiksacija zadnjeg ukrštenog ligamenta. Trogodišnji postoperativni period praćenja pokazao je intaktnu arterijsku perfuziju i veoma dobru funkciju kolena sa neznatnom razlikom u odnosu na suprotno koleno. **Zaključak.** Komplikovane ortopedske i vaskularne povrede kod dece su veoma retke i zahtevaju multidisciplinaran pristup.

Ključne reči:
koleno, povrede; a. poplitea; krvni sudovi, povrede; deca; hirurgija, vaskularna, procedure; graftovi; funkcija, povratak.

Introduction

Vascular injuries (especially blunt trauma) in infants and children are rare, occurring for less than 1% of pediatric trauma according to literature data [1]. However, the amputation rate associated with vascular injury, do not differ between adults and children. At the same time, pediatric patients showed an improved adjusted mortality when compared to adults [1]. The long term patency after repair of injured vessels, in children is not well documented in the literature. Because of that vascular trauma in infant and children age, is important clinical entity.

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Blunt trauma of the popliteal artery can be caused by many different mechanisms, including a posterior knee joint dislocation. These combined injuries are very rare in children. We presented such a case and reviewed the literature.

Case report

A 14-year-old boy was addmited urgently after motorcycle accident. Physical examination revealed sweeling and hemathoma over the right popliteal fossa associated with minimal knee deformity. The pedal pulses were absent. Ancle brachial indexes at both dorsalis pedis and posterior tibial arteries were less than 0.4. The patient underwent duplex ultrasonography which showed popliteal artery thrombosis, while popliteal vein was patent. The right femoral arteriography revealed occluded popliteal artery at the level of the knee joint (Figure 1).

Fig. 1 – A right femoral arteriogram demonstrating complete occlusion of the popliteal artery at the level of the knee joint. The crural arteries were patent.

The urgent surgical procedure under general anestesia was performed about four hours after injury. The dorsal approach to the popliteal artery revealed the rupture of the posterior cruciate ligament associated with advential hemorrhage and thrombosis through a 4.5 cm segment of popliteal artery (Figure 2a). A thrombosed segment of the popliteal artery was resected and replaced with a saphenous vein graft (Figures 2b and c). The resected part of the popliteal artery showed complete intimal disruption with secondary thrombosis (Figure 2d).

A transosseus femoral fixation of the posterior cruciate ligament using non-absorable stitch was carried out. Postoperatively, the patient had palpable both pedal pulses, while ancle brachial index was 1.0. Control arteriography demonstrated a patent graft with good distal runoff (Figure 3). There was no clinical signs of compartment syndrome and fasciotomy was unnecessary since there was no prolonged ischemia of lower limb.

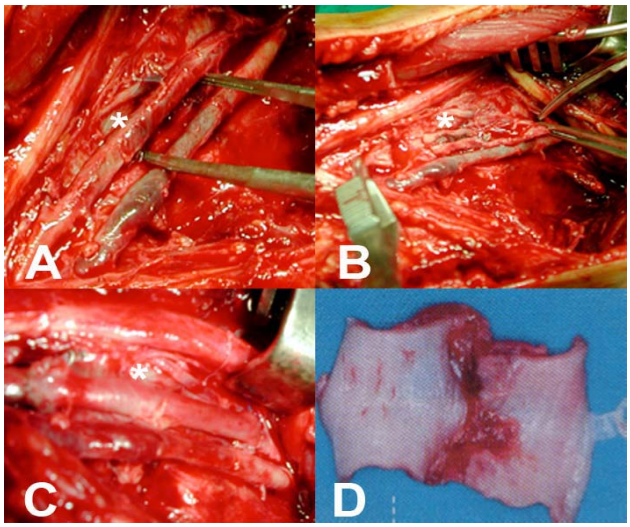

Fig. 2 – A) The adventitial hemorrhage and thrombosis of the popliteal artery (asterix); B) Following opening of the damaged artery the secondary thrombus (asterix) was found; C) The complete replacement with autologous saphenous vein graft (asterix); D) The intimal disruption of the damaged popliteal artery following removal of the secondary thrombus.

Fig. 3 – The white arrows demonstrating patent saphenous vein graft on control angiography.

Physiotherapy started with partial weight-bearing a week postoperatively. Six weeks later the patient started with full weight bearing. After 3 years, a follow up revealed that the patient had vascular graft with palpable pedal pulses and normal ancle brachial indices. No degenerative changes could be observed on radiography at the knee, while MRI confirmed intact both posterior and anterior cruciate ligament. There were no significant differences in functional statements between the left and right knee.

Discussion

Traffic accidents (injuries related to motor vehicle and motorcycle accidents) and sports activities (skiing, football) are the main reasons for knee injuries associated with posterior knee joint dislocation [2–4]. Falls from height are the second most common cause of knee dislocations [5]. Other interesting reported causes of knee dislocations include martial arts injury [6, 7], trampoline injuries [8], and spontaneous knee dislocation in the morbidly obese [9]. Male children have higher injury rates as a result of their more aggressive behavior and exposure to contact sports.

One of the most important causes of the posterior knee dislocation is a rupture of posterior cruciate ligament with or without proximal avulsion fracture [2, 10–15]. The posterior cruciate ligament is approximately twice as strong as that anterior cruciate one. Because of that, posterior cruciate ligament plays an important role in stabilizing the knee joint [16]. Injuries of the knee ligaments in children are rare [17, 18].

Fractures around the knee result in vascular injuries in about 3% of cases. However, the incidence or vascular events is about 16%, when the posterior knee dislocation is present [2–4, 13, 19]. The mean reason, regardless of the mechanism of injury or direction of dislocation, lies in the neurovascular anatomy of the knee. The popliteal vessels, run posteriorly within the popliteal fossa. The popliteal artery is fixed proximally at the adductor magnus hiatus, and distally at the fibrous arch of the soleus and interosseus membrane. Because of its "fixed" anatomical position, popliteal artery is predisposed to injury with posterior knee dislocation [4]. When posterior knee dislocation occurs, the popliteal artery can be stretched, lacerated, kinked, contused (followed by secondary arterial thrombosis) [2, 4, 14, 20], and even, transsected [14, 19, 21].

Injury to the popliteal artery predisposes to limb-threatening ischemia, although up to one third of patients may present with intact distal pulses [22]. The problem is how to recognize and avoid missing a popliteal artery injury in such cases. Is arteriography neccessary in all patients with posterior knee dislocation? Some authors recommend arteriography, only when physical signs (absent pedal pulses) or/and diminished ancle brachial indices, show arterial flow disturbance [19, 21, 23].

Pediatric vascular injury is uncommon during childhood and adolescence. Because of that, its surgical management is traditionally based on adult trauma experience. The first question is surgical approach. The popliteal artery reconstruction can be approached medially or posteriorly. The medial approach is preferable if distal thrombectomy is necessary, while the posterior approach may be helpful when treating short popliteal artery injuries. The stretch injury of the popliteal artery associated with intimal separation requires an abundant arterial resection before reconstruction [4, 20, 21]. Inadequate debridement of contused popliteal artery is always results in arterial thrombosis in the early postoperative period [24]. Vascular repair includes primary end-to-end anastomosis, vein graft interposition, or bypass grafting [4]. The majority of popliteal artery injuries (always when the length of damaged segment is more than 1.5 to 2 cm) secondary to knee dislocation, require an interposition vein graft secondary to the extent of arterial injury. End-to-end repair may require extensive popliteal artery mobilization with sacrifice of collateral vessels to ensure a tension-free repair [25].

However, in the pediatric population, there are same specific factors that should be respected when vascular traumatic lesions are treated. They are small vessel size, vessel spasm, tendency for restenosis, and rapid body growth. Most authors recommend to avoid the use of continuous sutures, because of possible narrowing along the suture with growth [26, 27]. Next, what happens with saphenous vein in arterial position several years after the reconstruction? On the basis of previous reports [28, 29], the risk of significant aneurismal dilation or occlusion of saphenous vein grafts is acceptable in children, even with long-term follow-up. So, autlogous saphenous vein is the material of choice for arterial reconstruction in children (Table 1).

Table 1

Early results and types of surgical procedures by various authors

The authors and the year	Sex (M/F)	Age (years)	Vascular procedure	Early result
Kirby et al, 1999 [4]	M	9	Reverse saphenous vein graft	Good
Dalsing et al, 2005 [29]	M	9	Reverse saphenous vein graft	Good
Angiletta et al, 2006 [31]	M	13	Stenting	Good
Lineena et al, 2008 [30]	M	11	Reverse saphenous vein graft	Good
	M	11	End-to-end	Good

M – male; F – female.

Few years ago stenting was described as a treatment of femoro-popliteal arterial trauma [30]. According to the author opinion the rationale for choosing stenting *vs* direct vascular reconstruction was based on the arterial caliber (approximately 4 mm) and the size of the great saphenous vein (approximately 2 mm), which led to the decision to defer further surgery until the child was older and the arteries and veins for reconstruction grew larger [28]. In our opinion this kind of injured popliteal artery treatment has some major disadvantages. There are stent fracture and migration as a result of biomechanical forces, as well as in-stent restenosis due to the healing process that induces neointimal hyperplasia. Because of that stenting could be eventually considered to be the bridge procedure to delay open surgery in smaller children.

Conclusion

Traumatic posterior knee dislocation is very often followed by popliteal artery damage. The standard Doppler ultrasound or multislice computed tomography angiography (DSA or MSCTA) are neccessary when pedal pulses are absent or/and ancle brachial indices significantly diminished. Such injuries should be treated interdisciplinary by the well-trained vascular surgeon and pediatric orthopedic surgeon.

Acknowledgments

The presented study was a part of scientific research project (No 175008) supported by the Ministry of Education, Science and technological Development of the Republic of Serbia.

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Received on October 3, 2012.
Revised on December 25, 2012.
Accepted on January 9, 2013.

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Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** i **literatura.**

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a) Naslov treba da bude kratak, jasan i informativan i da odgovara sadržaju rada. Podnaslove treba izbegavati.

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2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **uvod i cilj** rada, osnovne procedure - **metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi - **rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt (**250** reči) ima podnaslove: *uvod/cilj, metode, rezultati* i *zaključak*. Za apstrakte na engleskom dozvoljeno je i do **450** reči. Strukturisani apstrakt je obavezan za metaanalize (istog obima kao i za originalne članke) i kazuistiku (do 150 reči, sa podnaslovima *uvod, prikaz slučaja i zaključak*). Ispod apstrakta, pod podnaslovom „Ključne reči“ predložiti 3–10 ključnih reči ili kratkih izraza koji oslikavaju sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Zaključak** može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije. U **uvodu** ponovo napisati naslov rada, bez navođenja autora. Navesti hipotezu (ukoliko je ima) i ciljeve rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo strogo relevantne podatke iz literature i ne iznositi opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

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

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Primeri referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

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

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 fusnoti, ne u zaglavlju. Za fus-notu koristiti sledeće simbole ovim redosledom: ***, †, ‡, §, ||, ¶, **, ††, ...** Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

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Parts of the manuscript are: **Title page; Abstract with key words; Text; References**.

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The second page should carry a structured abstract with the title for original articles, metanalyses and case reports. The abstract should state the purposes of the study or investigation, basic procedures (selection of study subjects or laboratory animals; observational and analytical methods), main findings (giving specific data and their statistical significance, if possible), and the principal conclusions. It should emphasize new and important aspects of the study or observations. S t r u c t u r e d abstract should contain typical subtitles: *background/aim, methods, results* and *conclusion*. The abstract for metaanalyses and obrginal papers should have up to 450 words, and up to 150 words for case reports (with subtitles *background, case report, conclusion*). Below the abstract authors should provide, and identify as such, 3–10 key words or short phrases that will assist indexers in cross-indexing the article and will be published with the abstract.

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The text of original articles is divided into sections with the headings: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

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Discussion is to emphasize the new and important aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

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Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

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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