YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2011; July Vol. 68 (No. 7): p. 535-630.

YU ISSN 0042-8450 vol. 68, br. 7, 2011.

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, tehnički sekretar 3608 943, redakcija 3609 479 i 3609 179, pretplata 3608 997. Faks 2669 689. E-mail (redakcija): **vsp@vma.mod.gov.rs** i **vmavsp@hotmail.com**.

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 kod Uprave za javna plaćanja u Beogradu br. 840-941621-02 – VMA (za Vojnosanitetski pregled), PIB 102116082. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. 3608 997. Godišnja pretplata: 4 000 dinara za građane Srbije, 8 000 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. 68 No. 7, 2011

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: +381–11–3608–943; +381–11–3609–479, subscription 3608–997; Fax: +381–11–2669–689; E-mail: **vsp@vma.mod.gov.rs** and **vmavsp@hotmail.com**

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: Account in Uprava za javna plaćanja in Belgrade. Giro Account No. 840-941621-02 – VMA (za Vojnosanitetski pregled), PIB 102116082. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 4,000.00 Din, institutions 8,000.00 Din in Serbia, and foreign subscribers 150 €.

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

S A D R Ž A J / C O N T E N T S

ORIGINALNI ČLANCI / ORIGINAL ARTICLES

Milan Milovanović – Plava vrata (ulje na platnu, 480 × 390 mm), 1917. god. (Narodni muzej, Beograd)
Milan Milovanović (Kruševac, 1876–Beograd, 1946), jedan od istaknutih srpskih slikara s kraja 19. i početka 20. veka, kao učesnik u Prvom svetskom ratu svojim kistom ovekovečio je mnoga lica i događaje iz tog perioda. Slika „Plava vrata“ iz 1917. godine nastala je u Italiji (Kapri) gde se Milovanović oporavljao od tifusa koji je odneo mnoge živote među srpskim vojnicima tokom Prvog svetskog rata (vidi str. 621–5).

Milan Milovanović – The Blue Door (oil on canvas, 48 × 39 cm), 1917 (National Museum, Belgrade)
Milan Milovanović (Kruševac 1876 – Belgrade 1946) is one of outstanding Serbian artists from the end of XIX to the beginning of XX Century, who as the participant of World War I immortalized by his paintbrush numerous faces and events from that period. „The Blue Door“, oil on canvas, 1917, was painted in Italy (Capri) where Milovanović was to recover from typhus, the illness which took away many lives of Serbian soldiers during World War I (see page 621–5).

THE FIRST IMPACT FACTOR
OF THE *VOJNOSANITETSKI PREGLED*

0.199

(The 2010 Journal Citation Reports®, Thomson Reuters, 2011)

Dragi autori i čitaoci,

Veliko mi je zadovoljstvo što vas mogu obavestiti da je naš i vaš *Vojnosanitetski pregled* dobio svoj prvi impakt faktor (The 2010 Journal Citation Reports®, Thomson Reuters, 2011). On iznosi 0,199 i važan je pokazatelj interesovanja koje autori i čitaoci poklanjaju sadržaju *Vojnosanitetskog pregleda*.

Koristim ovu priliku da se još jednom zahvalim našim čitaocima, autorima, recenzentima, članovima uređivačkog odbora i redakcije časopisa na velikom angažovanju, tokom svih prethodnih godina, na podizanje kvaliteta i međunarodnog ugleda časopisa,

Iskreno verujem da će našim zajedničkim radom, u godinama koje slede, impakt faktor *Vojnosanitetskog pregleda* biti sve veći i veći!

Srdačno,
prof. dr Silva Dobrić
glavni i odgovorni urednik

Dear authors and readers,

It is my great pleasure to inform you that our and your *Vojnosanitetski pregled* has obtained the first impact factor: 0.199 (The 2010 Journal Citation Reports®, Thomson Reuters, 2011). Its value is an important indicator of the intrest the authors and readers place on the *Vojnosanitetski pregled* content.

On this occasion I would like to thank once again to our readers, authors, reviewers, and members of Editorial Board and Editorial Staff of the Journal for great efforts they have made in increasing its quality and international reputation in preceding years.

I truly believe that our further cooperation will result in higher and higher impact factor of the *Vojnosanitetski pregled* in the years, that are coming.

Cordially,

Prof. Silva Dobrić
Editor-in-Chief

ORIGINALNI ČLANCI

UDC: 57.084::612.22]::[615.835:616.155.1-092.9
DOI: 10.2298/VSP1107539B

Uticaj toksičnog dejstva kiseonika na membranu eritrocita i mogućnost procene poremećaja funkcije centralnog nervnog sistema

Impact of oxygen toxic action on the erythrocyte membrane and possibility of estimating central nervous system function disturbances

Branislava Belić*, Marko R. Cincović†

***Zavod za transfuziju krvi Vojvodine, Novi Sad, Srbija; †Poljoprivredni fakultet,**
Departman za veterinarsku medicinu, **Novi Sad, Srbija**

Apstrakt

Uvod/Cilj. Produženo izlaganje hiperbaričnom kiseoniku dovodi do promene oblika eritrocita zbog toksičnog delovanja kiseonika na njihovu membranu. Cilj ovog rada bio je da se ispita veza između pojave patoloških oblika eritrocita u različitim vremenskim periodima od početka izlaganja hiperbaričnoj oksigenaciji i momenta nastanka konvulzije, zatim međusobna veza zastupljenosti različitih patoloških oblika eritrocita tokom izlaganja hiperbaričnoj oksigenaciji, kao i veza između zastupljenosti rupturisanih eritrocita i funkcije centralnog nervnog sistema (CNS) posle završenog hiperbaričnog tretmana. **Metode.** Šezdeset laboratorijskih miševa soja *Mus musculus* izlagano je 100% kiseoniku pod pritiskom od 3,5 apsolutnih atmosfera (ATA). Životinjama je krv uzimana iz repne vene u 32, 34, 36, 38. i 40. minutu od početka hiperbarične oksigenacije, a prisustvo patoloških oblika eritrocita ispitivano je pomoću elektronskog mikroskopa. Tokom trajanja hiperbarične oksigenacije beleženo je vreme nastanka konvulzija kod ekperimentalnih životinja. Posle dekompresije vršen je neurološki pregled životinja i registrovano je prisustvo (ocena 1), odnosno odsustvo (ocena 0) određenih refleksa. Izračunate su jednačina linearne regresije i Pearson-ov koeficijent korelacije za parametre navedene u ciljevima rada. **Rezultati.** Hiperbarični kiseonik doveo je do oštećenja eritrocita već u 34. minutu od početka tretmana uz pojavu različitih patoloških oblika eritrocita. Neposredno pred pojavu ireverzibilnih promena (ruptura membrane eritrocita) najzastupljeniji oblik patološki izmenjenih eritrocita bili su ehinociti. Broj oštećenih eritrocita u 34. minutu statistički je značajno korelisao sa stepenom oštećenja eritrocita u kasnijim vremenskim intervalima (36, 38. i 40. minutu od početka hiperbarične oksigenacije). Takođe, broj oštećenih eritrocita bio je u korelaciji i sa pojavom konvulzija. Naime, što je broj oštećenih eritrocita bio veći, konvulzije su se pojavljivale značajno ranije kod ispitivanih jedinki ($p < 0,01$). Utvrđena je i negativna korelacija između broja ireverzibilno oštećenih eritrocita (ruptura) u 40. minutu i neurološkog skora kod ispitivanih životinja ($p < 0,05$). **Zaključak.** Analizom broja izmenjenih eritrocita tokom hiperbarične oksigenacije može se predvideti momenat nastanka konvulzija, a samim tim i ograničiti trajanje oksigenacije. Pojava ehinocita ukazuje na skoru rupturu eritrocita i mogući nastanak konvulzija. Broj rupturisanih eritrocita može ukazati na potencijalno oštećenje funkcije CNS-a i posle prestanka izlaganja kiseoniku pod pritiskom.

Ključne reči:
hiperbarička oksigenacija; nervni sistem, centralni; konvulzije; toksičnost, akutna, testovi; eritrociti; miševi.

Abstract

Background/Aim. Prolonged exposure to hyperbaric oxygen leads to changes of erythrocytes shape as a consequence of toxic effects of oxygen on the erythrocyte membrane. The aim of this study was to examine the association between occurance of pathological forms of erythrocytes at different time from the start of hyperbaric oxygenation and the moment of convulsions occurrence, an interrelationship of different pathological forms of erythrocytes during exposure to hyperbaric oxygenation, as well as the correlation between the presence of ruptured erythrocytes and function of central nervous system (CNS) after completion of hyperbaric treatment. **Methods.** Sixty laboratory mice, *Mus musculus*, were exposed to the wholly-oxygen pressure of 3.5 absolute atmospheres (ATA). Blood was collected at the 32nd, 34th, 36th, 38th and 40th minutes after the exposure to oxygen. Pathological forms of erythrocytes were examined by electron microscopy. A moment of convulsions occurrence was registered in all animals. After decompression neurological examinations of experimental animals were perfomed. The Pearson's coefficient of correlation, and linear regression equations for the parameters outlined in the aim of the study were calculated. **Results.** Hyperbaric oxy-

Correspondence to: Branislava Belić, Skupština AP Vojvodine, Vladike Platona bb, 21 000 Novi Sad. Tel.: +381 21 4874 113, +381 63 66 91 25, Fax: +381 21 456 755. E-mail: bbelic@skupstinavojvodine.gov.rs

gen caused damages of erythrocytes at the 34th minute after beginning of the treatment. Various forms of abnormal red blood cells occured, and immediately before the occurrence of irreversible changes (erythrocyte membrane rupture) echinocyte shape was dominated. A significant correlation between the number of damaged red blood cells at 34th minute and their number at the 36th, 38th and 40th minute was found. Convulsions were diagnosed significantly earlier in mice with a greater number of damaged red blood cells ($p < 0.01$). There was a negative correlation between the number of irreversiblly damaged red blood cells (ruptured) at the 40th minute and neurological score in the studied animals ($p < 0.05$). **Conclusion.** The analysis of altered erythrocytes during hyperbaric oxygenation could predict a moment of seizures occurrence, and therefore the duration of the therapy with hyperbaric oxygen. Ehinocytes indicate impending rupture of red blood cells and a possible occurrence of seizures. An increased number of ruptured red blood cells may also even indicate the potential burden of CNS after cessation of hyperbaric oxygenation.

Key words:
hyperbaric oxygenation; central nervous system; seizures; acute toxicity tests; erythrocytes; mice.

Uvod

Hiperbarična terapija prvi put je opisana u XVII veku naše ere, kada se pominje hiperbarična komora zvana domicilium. U toku XIX veka javljaju se pretpostavke toksičnog dejstva kiseonika u stanjima produžene anestezije [1]. Krajem XIX i početkom XX veka dolazi do usavršavanja mikroskopskih tehnika, uključujući i elektronsku mikroskopiju, koja je pomogla da i ovaj eksperimentalni rad, koji je imao cilj da rasvetli neka zbivanja vezana za morfologiju eritrocita pod uticajem hiperbarične oksigenacije, bude doveden do kraja.

Eritrocit ili crvena ćelija krvi je funkcionalno zrela ćelija periferne krvi, specifičnog oblika, prilagođena prenosu kiseonika iz pluća u periferna tkiva, da bi se zadovoljile potrebe organizma za kiseonikom. Kiseonik je neophodan za stvaranje energije, čime se reguliše i održava funkcionalna sposobnost i unutrašnja ravnoteža ćelije. Transport kiseonika je biohemijski aktivan proces [2, 3]. Prolongirano izlaganje hiperbaričnom kiseoniku dovodi do promene oblika eritrocita zbog toksičnog delovanja kiseonika na membranu eritrocita. U prethodnim ispitivanjima utvrđeno je da postoji veza između intenziteta promena oblika eritrocita i momenta nastanka konvulzija tokom izlaganja hiperbaričnoj oksigenaciji [4]. Tako je pokazano da prilikom izlaganja eksperimentalnih životinja hiperbaričnom kiseoniku od 3,5 apsolutnih atmosfera (ATA) u 40. minutu najveći broj životinja ispolji konvulzije i da tada dominiraju patološke forme eritrocita (80–90%).

Cilj ovog rada bio je da se detaljnije ispita veza između pojave patoloških oblika eritrocita u različitim vremenima od početka izlaganja hiperbaričnoj oksigenaciji i momenta nastanka konvulzija, zatim međusobna veza zastupljenosti različitih patoloških oblika eritrocita tokom izlaganja hiperbaričnoj oksigenaciji, kao i veza između zastupljenosti rupturisanih eritrocita i funkcije centralnog nervnog sistema (CNS) posle završenog hiperbaričnog tretmana.

Metode

Eksperimenti su vršeni na laboratorijskim miševima (*Mus musculus*) starosti 3–5 nedelja, koji su gajeni pod istim uslovima ishrane i nege. Ova vrsta posebno je prilagođena za rad u laboratorijskim uslovima, a poznata je njihova otpornost i visoki nivo preživljavanja, čak i posle ozbiljnijih operacija. Životinje su bile podeljene na eksperimentalnu i na kontrolnu grupu, po 60 jedinki u svakoj.

Životinje iz eksperimentalne grupe bile su stavljane u hiperbarične komore, posebno namenjene za eksperimentalna istraživanja (tip Dräger 1 200). Životinje iz kontrolne grupe boravile su u komorama, ali nisu izlagane hiperbaričnom kiseoniku.

Komora ima performanse koje omogućuju dostizanje pritiska do 40 ATA. Ovaj tip komore omogućava i stalno praćenje važnih parametara kao što su: EKG, EEG, vrednost parcijalnog pritiska kiseonika, kao i njegove promene. Na komori se nalaze i izvodi za uzorkovanje krvi tokom trajanja tretmana.

Posle hermetizacije počinjalo se sa kompresijom (0,2 bara/minut) koja je kod eksperimentalnih životinja išla do 3,5 ATA. Vreme ekspozicije životinja bilo je oko 40 minuta. Temperatura u komori bila je 37ºC, a vlažnost vazduha između 60 i 70%. Navedene vrednosti po standardima predstavljaju zonu komfora u ovim uslovima. Posle ekspozicije, interval dekompresije trajao je oko 30 minuta.

Uzorci krvi uzimani su od eksperimentalnih životinja direktno iz repne vene. Toksični efekti kiseonika na membranu eritrocita posmatrani su elektronskim mikroskopom, a priprema preparata vršena je prema ranije opisanim postupcima [5, 6].

Krv je uzimana u 32, 34, 36, 38. i 40. minutu od početka izlaganja hiperbaričnim uslovima. Ovo vreme izabrano je na osnovu ranije ispitane pojave nastanka konvulzija kod laboratorijskih pacova [4], koje predstavljaju kontraindikaciju za dalje izlaganje hiperbaričnim uslovima.

Neurološko ispitivanje CNS-a podrazumevalo je, osim praćenja nastanka konvulzija, sledeće: refleks fleksije, refleks hvatanja, refleks ispravljanja, izazivanje reakcije, test ekvilibrijuma, kornealni refleks, pupilarni refleks, reakciju na auditorni stumulus, refleks odmahivanja glavom, refleks širenja prstiju, što je modifikovano prema Tupper-u i Wallace-u [7]. Postojanje aktivnosti bilo je ocenjeno sa 1, dok je odložena ili spora aktivnost, kao i odsustvo aktivnosti ocenjeno sa 0.

Statistička obrada dobijenih podataka podrazumevala je procenu povezanosti broja izmenjenih eritrocita i momenta nastanka konvulzija, međusobnu povezanost broja i vremena nastanka različitih patoloških oblika eritrocita, kao i povezanosti broja rupturisanih eritrocita i skora neuroloških ispiti-

vanja posle završetka hiperbarične oksigenacije. Statistička značajnost ovih korelacija određivana je pomoću jednačina linerane regresije i Pearson-ovog koeficijenta korelacije. U statističkoj obradi podataka korišćen je statistički paket Statistica 5 (Stat. Soft. Inc.).

Rezultati

Kiseonik pod pritiskom od 3,5 ATA doveo je do pojave patoloških formi eritrocita već u 34. minutu od početka izlaganja životinja hiperbaričnoj oksigenaciji. Broj nepromenjenih eritrocita značajno je opadao tokom trajanja hiperbarične oksigenacije, tako da u 40. minutu od početka tretmana bilo ih je svega 10–20% (tabela 1). Među patološkim oblicima eritrocita registrovani su akantociti, stomatociti, ehinociti i eritrociti sa rupturisanom membranom. Oni su se pojavljivali upravo ovim redosledom, tako da je poslednja forma koja je dominirala pred nastanak rupture eritrocita bila forma ehinocita. S druge strane, kod kontrolne grupe životinja, svi eritrociti bili su nepromenjenog oblika (slika 1).

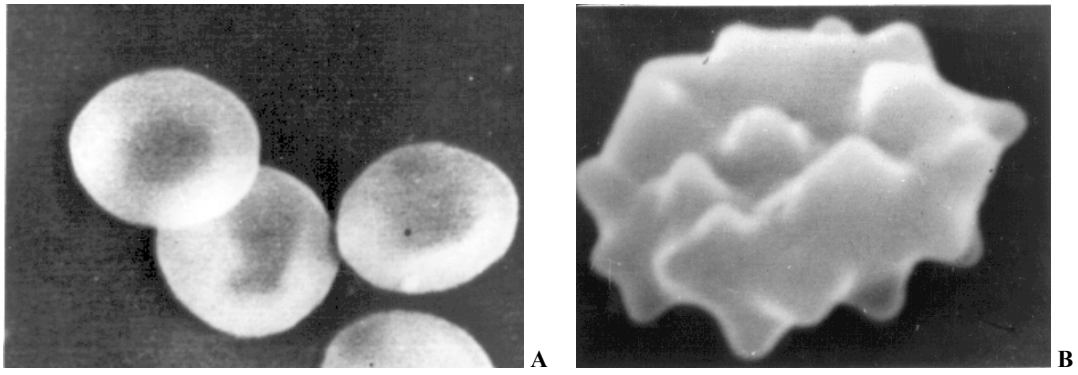
Rezultati prikazani u tabeli 2 pokazuju da se na osnovu rane pojave patoloških oblika eritrocita može sa visokom značajnošću predvideti pojava drugih patoloških oblika ovih ćelija, pa čak i rupturisane forme eritrocita u daljem toku izlaganja hiperbaričnom kiseoniku. Ovo je posebno interesantan nalaz, ako se uzme u obzir da se ruptura eritrocita i konvulzije dešavaju u vrlo uskom vremenskom intervalu, pa je na osnovu pojave ranih patoloških formi eritrocita moguće znatno pre pojave konvulzija predvideti njihovo ispoljavanje. Ipak, ovi rezultati zahtevaju obimnija istraživanja na većem broju životinja, uz učestalije merenje. Kada se uporedi jačina korelacije između različitih oblika eritrocita uočava se da najveća korelacija postoji između akantocita i stomatocita, stomatocita i ehinocita, kao i ehinocita i rupturisanih eritrocita. Ovakav nalaz, preslikan na kliničke uslove, sugeriše da pojava ehinocita kod bolesnika tokom hiperbarične oksigenacije može ukazati na njihovu osetljivost prema kiseoniku pod pritiskom.

Broj oštećenih eritrocita tokom hiperbarične oksigenacije bio je značajno povezan sa vremenom nastanka konvul-

Tabela 1

Zastupljenost patoloških oblika eritrocita (E) kod miševa u 40. minutu od početka izlaganja životinja hiperbaričnoj oksigenaciji (HO)

Vreme od početka HO (min)	Oblici eritrocita (%)				
	Akantocit	Stomatocit	Ehinocit	Rupturisani E	Normalni E
32	0	0	0	0	100
34	1–2	0	0	0	98–99
36	10–15	20–30	0	0	55–70
38	10	10	40–45	0	35–40
40	0	0	55–60	25–30	10–20



A **B**

Sl. 1 – Izgled nepromenjenog eritrocita (A) i ehinocita (B) čija pojava prethodi rupturi membrane eritrocita

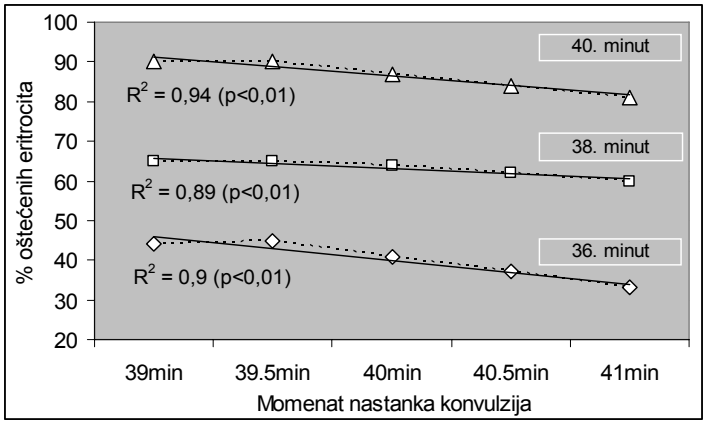
Tabela 2

Ispitivanje korelacije zastupljenosti patoloških oblika eritrocita (E) u krvi miševa u različito vreme od početka izlaganja životinja hiperbaričnoj oksigenaciji

Oblici eritrocita	36. min akantociti	36. min stomatociti	38. min ehinociti	40. min ehinociti	40. min rupturisani E
36. min akantociti	1	0,85**	0,74**	0,65*	0,59*
36. min stomatociti		1	0,91**	0,71**	0,66*
38. min ehinociti			1	0,89**	0,74**
40. min ehinociti				1	0,92***
40. min upturisani E					1

***p* < 0,1 ****p* < 0,01 *****p* < 0,001

zija (slika 2). Što je broj oštećenih eritrocita bio veći, konvulzije su se ranije javljale. Ako bi broj oštećenih eritrocita bio nezavisno promenljiva, a vreme pojave konvulzija zavisno promenljiva, formule linearne jednačine za 36, 38. i 40. minutu ekspozicije hiperbaričnoj oksigenaciji bio bi, redom: y_{36} = 46-0,15x ; y_{38} = 61,851-0,3457x ; y_{40} = 56,941-0,1961x. Pored ovoga, povišen broj rupturisanih eritrocita bio je indikator eventualne kompromitovanosti CNS-a, budući da je bio u značajnoj negativnoj korelaciji sa neurološkim skorom životinja izloženih dejstvu kiseonika pod pritiskom ($p < 0,05$) (slika 3). Najčešće izostali refleksi bili su: refleks hvatanja, ekvilibrijuma, refleks posle izazivanja reakcije, refleks odmahivanja glavom i refleks širenja prstiju.



Sl. 2 – Pojava konvulzija kod miševa u funkciji broja izmenjenih oblika eritrocita u 36, 38. i 40. minutu od početka izlaganja životinja hiperbaričnoj oksigenaciji

Diskusija

Naši rezultati pokazali su da tokom izlaganja eksperimentalnih životinja hiperbaričnom kiseoniku dolazi do promena u obliku eritrocita, pa čak i do pojave ruptura u njihovoj membrani, što predstavlja znak njihovog ireverzibilnog oštećenja. Pojava rupturisanih eritrocita značajno koreliše sa pojavom konvulzija kod životinja izloženih dejstvu kiseonika pod povišenim pritiskom. Budući da je ustanovljena značajna povezanost između pojave pojedinih patoloških formi eritrocita u zavisnosti od trajanja hiperbarične oksidacije, moguće je na osnovu ranih promena u obliku eritrocita predvideti pojavu ozbiljnijih oštećenja ovih ćelija, kao i funkcije CNS-a, što može da ima i praktične implikacije u smislu planiranja trajanja ovakvog tretmana.

Kiseonik, u koncentraciji nižoj od 60% i pod pritiskom od jedne atmosfere ne izaziva toksične efekte. Međutim, pod pritiskom od jedne atmosfere kiseonik se može udisati samo tokom četiri časa bez štetnog dejstva, pod uslovom da ga udiše zdrava, odrasla osoba. Vreme njegovog udisanja bez izazivanja oštećenja skraćuje se sa porastom pritiska. Tako npr, ogranizam može da udiše kiseonik pod pritiskom od 2 ATA tokom tri časa, a dva časa ako je pod pritiskom od 3 ATA. Najvažnije je davati kiseonik samo onoliko koliko je potrebno, jer je u većim količinama toksičan za svaku živu ćeliju. Simptomatologija trovanja kiseonikom je veoma kompleksna zbog čestog preplitanja simptoma. Prilikom trovanja kiseonikom dolazi do usporavanja oksidacije glukoze, fruktoze i laktoze, sputavanja hemoreceptora u aorti i *glomus coraticum-u*, povećanja tonusa *nervus vagus-a,* smanjenja minutnog volumena srca, dilatacije krvnih sudova pluća, konstrikcije cerebralnih i renalnih krvnih sudova, pojave tonično-kloničnih konvulzija, plućnog edema, atelektaza i alveolokapilarne blokade [8].

Toksični efekat kiseonika na CNS naziva se „Bert-ov efekat“, a ime je dobio po Paul-u Bert-u, koji je 1878. godine demonstrirao konvulzije kod ptica izloženih pritisku od 15-20 ATA (apsolutni pritisak vazdušnog omotača). Takozvani „Smith-ov efekat“ predstavlja efekat kiseonika na plućni sistem, koji je dobio naziv po Lorain-u Smith-u, koji je 1899. godine opisao fatalnu pneumoniju kod pacova četiri dana posle izlaganja životinja 73% kiseoniku pod pritiskom od 1 ATA. Kod akutnog trovanja dominiraju simptomi vezani za CNS, dok kod hroničnog trovanja dominiraju pulmonalni simptomi [9]. Detaljnija istraživanja, pored manifestnih konvulzija, pokazala su, i promene u EEG zapisu [10]. Razloge pomenutih promena možemo naći u izmenjenoj cirkulaciji u CNS-u, promenama metabolizma NO i NADPH, promenama u citoskeletu usled oksidativnog stresa i dr [11].

Takve promene u eritrocitima mogu se javiti u različitim patološkim procesima, npr. u primarnim i sekundarnim anemijama, ali najčešće bez karakteristične rupture membrane eritrocita. U toksičnoj oksigenaciji rupture nastaju kao posledica specifičnog rasporeda fosfolipida, kada na mestima izmene zid eritrocita postaje umanjeno rigidan [12–14]. Pojava ruptura membrane eritrocita je ireverzibilna, a nastaje na mestu predhodnog istanjenja membrane [5, 6].

Dalja istraživanja iz ove oblasti treba usmeriti na detaljnija ispitivanja proteinske i lipidne strukture zida eritrocita i ispitivanje enzimske antioksidativne aktivnosti u krvi. U prilog navedenim činjenicama idu i novija istraživanja koja pokazuju da je oksidativni stres značajan patofiziološki mehanizam oštećenja eritrocita u hiperbaričnim uslovima [15].

Ovakva istraživanja mogu imati značaj za planiranje terapije hiperbaričnim kiseonikom u pogledu izbora odgovarajuće doze (pritiska kiseonika) i trajanja tretmana, jer će se na taj način dodatno poboljšati ekonomičnost lečenja i zaštita bolesnika od potencijalno toksičnog efekta kiseonika.

Zaključak

Analizom broja izmenjenih eritrocita tokom hiperbarične oksigenacije može se predvideti momenat nastanka konvulzija, a samim tim i ograničiti trajanje oksigenacije. Pojava ehinocita ukazuje na skoru rupturu eritrocita i mogući nastanak konvulzija. Broj rupturisanih eritrocita može ukazati na potencijalno oštećenje funkcije CNS-a i posle prestanka izlaganja kiseoniku pod pritiskom.

L I T E R A T U R A

1. *Gill AL, Bell CN.* Hyperbaric oxygen: its uses, mechanisms of action and outcomes. QJM 2004; 97(7): 385–95.
2. *Bruce LJ.* Red cell membrane transport abnormalities. Curr Opin Hematol 2008; 15(3): 184–90.
3. *Tomaszewski CA, Thom SR.* Use of hyperbaric oxygen in toxicology. Emerg Med Clin North Am 1994; 12(2): 437–59.
4. *Belić B, Cincović MR.* Comparative research of hyperbaric oxygen toxic effect to CNS. and erythrocytes Contemp Agricult 2009; 58(3–4): 52–7.
5. *Belić B, Cincović MR, Belić J, Vidović B.* Irreversible erythrocyte membrane rupture caused by hyperbaric oxygenation. Contemp Agricult 2010; 59(1–2): 151–5.
6. *Belić B.* The examination of the toxic effect of the oxygen on the erythrocyte membrane. Chronicle of scientific papers 2008; 32(1): 140–7. (Serbian)
7. *Tupper DE, Wallace RB.* Utility of the neurological examination in rats. Acta Neurobiol Exp (Wars) 1980; 40(6): 999–1003.
8. *Dekleva N.* Introduction in hyperbaric medicine. Beograd: Naučna knjiga; 1989. (Serbian)
9. *Dharmeskumar NP, Ashish G, Agarwal SB, Praveenkumar G, Krishna KL.* Oxygen Toxicity. J Indian Acad Clin Med 2003; 4(3): 234–7.
10. *Kurasako T, Takeda Y, Hirakawa M.* Increase in cerebral blood flow as a predictor of hyperbaric oxygen-induced convulsion in artificially ventilated rats. Acta Med Okayama 2000; 54(1): 15–20.
11. *D'Agostino DP, Colomb DG Jr, Dean JB.* Effects of hyperbaric gases on membrane nanostructure and function in neurons. J Appl Physiol 2009; 106(3): 996–1003.
12. *Byers TJ, Brandin E, Lue RA, Winograd E, Branton D.* The complete sequence of Drosophila beta-spectrin reveals supramotifs comprising eight 106-residue segments. Proc Natl Acad Sci U S A 1992; 89(13): 6187–91.
13. *Mische SM, Morrow JS.* Spectrin phosphorylation regulates spectrinsubunit interactions. J Cell Biol 1989; 107: 469a.
14. *Lambert S, Yu H, Prchal JT, Lawler J, Ruff P, Speicher D,* et al. cDNA sequence for human erythrocyte ankyrin. Proc Natl Acad Sci U S A 1990; 87(5): 1730–4.
15. *Oter S, Korkmaz A, Topal T, Ozcan O, Sadir S, Ozler M*, et al. Correlation between hyperbaric oxygen exposure pressures and oxidative parameters in rat lung, brain, and erythrocytes. Clin Biochem 2005; 38(8): 706–11.

Primljen 15. 12. 2009.
Revidiran 30.12. 2009.
Prihvaćen 19. 1. 2010.

ORIGINAL ARTICLE

UDC: 617.735:616.832-004
DOI: 10.2298/VSP1107544S

Retinal periphlebitis in patients with multiple sclerosis

Retinalni periflebitis kod bolesnika sa multiplom sklerozom

Miroslav Stamenković*, Dragana Obradović†

***University Medical Center Zvezdara,** Eye Clinic, **Belgrade, Serbia;**
†Military Medical Academy, Clinic for Neurology, **Serbia, Belgrade**

Abstract

Background/Aim. Multiple sclerosis (MS) is an immune-mediated disorder of the central nervous system (CNS), characterized by inflammation, demyelination and axonal loss. Retinal periphlebitis (RP) is often present in MS patients with similar evolution and histopathological changes as MS lesions. The aim of this study was to analyze the presence of RP in MS patients during different clinical phases, and its connection with impairment of blood-brain barrier. **Methods.** The study included 45 patients (26 females and 19 males) with MS. Their average age was 33.2 ± 8.1 years. There were 28 patients with relapsing-remitting (RR) form, 7 with primary progresive (PP) and 10 with secondary progressive (SP) form of MS. There were 27 patients in the relapse and 18 patients in the remission phase. The average MS duration was 7.48 ± 1.3 years. Ophthalmological, neurological and MRI examination were performed in all the patients, as well as cerebrospinal fluid sampling. Albumin ratio and IgG index were calculated in all the patients. **Results.** There were 9 patients with RP, and 36 without it. MS duration was significantly longer in the RP group. RP was much more common in the progressive form and was not present in the remission phase of MS. Albumin ratio values were increased in the group with RP. IgG index and IgG synthesis according to Tourtellotte formula, were statistically higher in the group of patients with RP. The values of visual evoked potentials (VEP's) latency were significantly higher in the group of patients with RP. **Conclusion.** The presence of RP is a reliable indicator of MS activity and might be considered as a parameter for monitoring the disease activity and effects of the treatment.

Key words:
multiple sclerosis; phlebitis; retinal diseases; diagnosis; prognosis.

Apstrakt

Uvod/Cilj. Multipla skleroza (MS) je imunološki posredovano oboljenje centralnog nervnog sistema (CNS) koje karakteriše inflamacija, demijelinizacija i gubitak aksona. Retinalni periflebitis (RP) je čest nalaz kod bolesnika sa MS i ima identičnu evoluciju i patohistološke promene kao MS lezija. Cilj ove studije bio je da se analizira prisustvo RP kod bolesnika sa MS tokom različitih kliničkih faza MS i njegova povezanost sa oštećenjem krvnomoždane barijere. **Metode.** U studiju bilo je uključeno 45 bolesnika sa MS (26 žena i 19 muškaraca). Prosečna starost ispitivane grupe bila je 33,2 ± 8,1 godine. Bilo je 28 bolesnika sa *relapsing-remitting* (RR) formom, sedam sa *primary progresive* (PP) i 10 sa *secondary progressive* (SP) formom MS. Bilo je 27 bolesnika u relapsnoj i 18 u remisionoj fazi. Prosečno trajanje MS iznosilo je 7,48 ± 1,3 godine. Kod svih bolesnika izvršeno je oftalmološko, neurološko i MRI ispitivanje, kao i ispitivanje uzoraka cerebrospinalne tečnosti. Albuminski odnos i IgG indeks izračunavani su kod svih bolesnika. **Rezultati.** Bilo je devet bolesnika sa RP i 36 bez njega. Trajanje MS bilo je duže u grupi bolesnika sa RP; RP je bio najprisutniji u grupi bolesnika sa progresivnom formom oboljenja i nije bio prisutan kod bolesnika sa RR formom. Vrednosti albuminskog odnosa bile su značajno veće u grupi bolesnika sa RP. Vrednosti IgG indeksa i intratekalne IgG sinteze računate prema Tourtellotte-ovoj formuli bile su značajno veće u grupi bolesnika sa RP; Gd+ lezije na MRI bile su značajno prisutnije u grupi bolesnika sa RP; vrednosti latence vizuelnih evociranih potencijala (VEP) bile su značajno veće u grupi bolesnika sa RP. **Zaključak.** Prisustvo RP predstavlja pouzdan indikator aktivnosti MS i može biti prihvaćen kao parametar za praćenje aktivnosti oboljenja i efekata primenjene terapije.

Ključne reči:
multipla skleroza; flebitis; mrežnjača, bolesti; dijagnoza; prognoza.

Introduction

Multiple sclerosis (MS) is an immune-mediated disorder of the central nervous system (CNS) which is characterized by inflammation, demyelination and axonal loss. The most common course is relapsing-remitting (RR) with unpredictable duration of remissions and frequency of relapses. In time, the majority of patients with RR form of MS turn into secondary progressive (SP) form, though in a smaller sample of patients the course might be primary progressive

Correspondence to: Miroslav Stamenković, University Medical Center Zvezdara, Eye Clinic, Dimitrija Tucovića 161, 11000 Belgrade, Serbia. Phone: +381 11 24 14 931. E-mail: drmiroslavstamenkovic@gmail.com

from the very beginning. MS is a disease of younger population, with age prevalence between 20 and 40 years. Females are more often affected and in time MS results in a severe neurological and functional deficit and disability [1].

The basic pathoanatomical findings are demyelinated plaques in white matter, especially periventicular localization, cervical spine and optical nerve. Disseminated plaques are infiltrates around blood vessels. Similar perivascular infiltrates are found around blood vessels of the retina, the most commonly around veins [2]. Rucker was the first to describe the changes in blood vessels in patients with multiple sclerosis. Today, it is believed that veins of retinal periphery are affected by the same pathological process as in MS [3–7]. Inflammation may be localized on the larger retinal veins as well. The disorder is characterized by focal and diffuse vein sheathing (Rucker's sign), sheathing centered on sites of arterio venous crossover, focal perivenous hemorrhage [8] and perivascular gliosis [9]. Active vascular disease is characterized by white exudates of round or oval contours around retinal veins resulting in white sheathing or cuffing of the affected vessels [10]. Blood vessels of retinal periphery show the sheathing, narrowing of lumens, while the inadequate perfusion of the retinal periphery induces the progress of degenerative changes in the periphery, appearance of retinoschisis, ruptures, thrombosis of blood vessels, consequently ishemia and neovascularisation in the occlusive forms of periphlebitis [11–13]. Retinal periphlebitis (RP) occurs in discrete episodes and often relapses, it is frequently bilateral, and may affect one or multiple retinal veins. The disorder of peripheral retinal blood vessels is not in correlation with the impairment degree of the optic nerve [14]. However, the disorder of blood vessels in peripapillar and perimacular region may cause the development of cystoid macular edema [15–17]. Cases of association of periphlebitis with anterior uveitis and cystoid macular edema have been described [18–21].The pathological substrate of retinal venous inflammation, i.e. RP is identical to the one existing in the CNS of MS patients which is characterized by perivascular infiltration of lymphocytes and plasmocytes [22–24]. The evolution of RP is relapsing, similar to the evolution of changes occurring in the CNS of MS patients [25–27]. Changes in retina, including inflammatory processes, may represent the indicators of generalized inflammatory response of the CNS during the course of MS.

There are few studies related to the presence of RP in MS patients [28–32]. However, to the best of our knowledge there is no data concerning correlation of RP and MS type, neurological disability and cerebrospinal fluid (CSF) parameters.

In regard to this, the aim of the study was to investigate the presence of RP in MS patients, to investigate the correlation between MS type and RP presence, as well as correlation between RP and disease severity, CSF and MRI parameters.

Methods

The study included 45 patients with the established diagnosis of MS according to Poser criteria [33]. All the patients were examined and treated in the Department of Neurology, Military Medical Academy, Belgrade. The patients were examined by the same neurologist, neurological exam was scored by using Expanded Disability Status Scale (EDSS) [34]. According to MS course, all three types of MS were present: RR, SP and PP. In relation to present disease activity, the patients were divided into the remission, relapse and progressive group. All the patients were examined by the same ophthalmologist for the presence of RP. The direct ophthalmoscopy (Goldmann's three-mirror lens) were used for diagnosing RP. According to the presence of RP (Figure 1), the patients were divided into two groups.

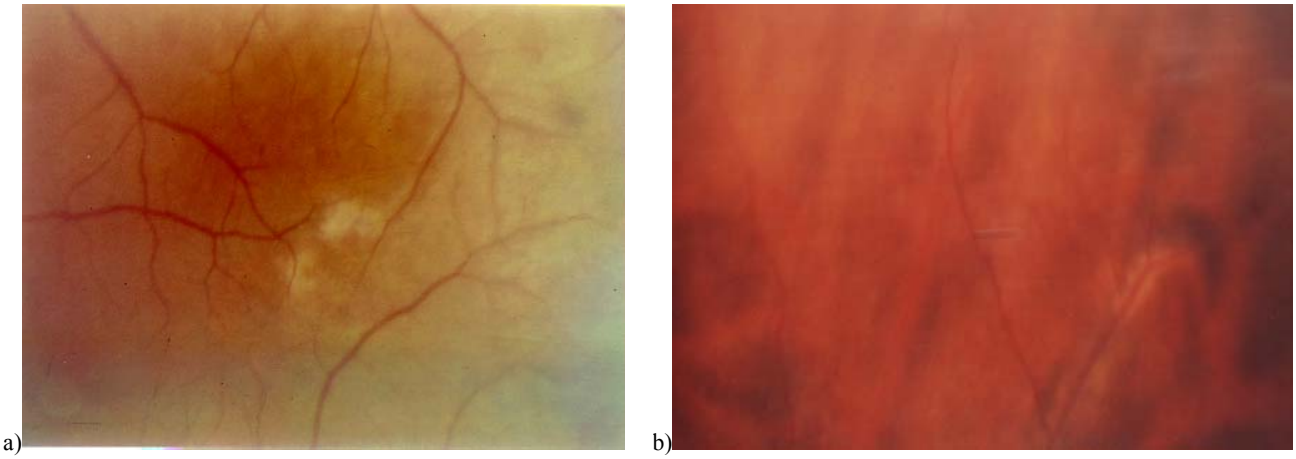


Fig. 1 – Retinal periphlebitis in patients with multiple sclerosis
a) focal and diffuse vein sheathing (Rucker's sign)
b) perivascular gliosis

CSF sampling was performed by lumbal puncture in all the patients. Following analyses were performed: CSF protein levels, CSF and serum albumin and IgG levels. Albumin ratio, a marker of blood-brain barrier damage, was calculated (cerebrospinal fluid albumins/serum albumins), normal range considered to be < 5.7.

In order to determine intrathecal IgG synthesis as a marker of immunological activity within the CNS, IgG index

was calculated [IgG CSF: IgG serum (albumin CSF/albumin serum)], as well as intrathecal IgG synthesis by using a modified Tourtellotte's formula [35].

Brain MRI was performed in all the patients within the three days of neurological and ophtalmological examination. It was performed on MRI General Electric 0.5T in the Department of Radiology, Military Medical Academy, Belgrade. In all the patients brain MRI scans were performed with and without Gadholinium (Gd) contrast according to standardized recommended procedure [36, 37]. The presence of Gd+ lesions on MRI scans was considered as marker of blood-brain barrier damage.

Visually evoked potentials (VEP) were performed in standard way [38, 39] in the Department of Neurophysiology, Military Medical Academy, Belgrade. According to our control group, amplitude values from 4.5 to 10 μV were considered normal while latency values from 90 to 110 ms were considered as normal. The values of the amplitude below normal were regarded as the parameter of disease activity.

The average and standard deviations were calculated for all parameters of observation, and the significance of differences was determined by Student's *t*-test. For non-parametric characteristics of observation, the frequency was registered, and the importance of differences was determined by χ^2 test. Regarding small specimens, Fischer's test of precise probability was used. In both cases if the value of *p* was less than 0.05, the difference was considered statistically significant.

Results

This study included 45 patients with the established diagnosis of multiple sclerosis (26 females and 19 males). The average age was 33.2 ± 8.17 years. In regard to MS type, there were 28 patients with RR form, 7 with PP and 10 with SP form of MS (Table 1). There were 27 patients in the relapse phase and 18 patients in the remission phase.

The average duration of MS was 7.48 ± 1.347 years in the study group.

On the basis of RP presence, all the patients were divided into two groups: with RP there were 9 patients, and without the RP 36 patients. MS duration was longer in the group of patients with RP (9.77 ± 1.81 years), than in the group without RP (6.91 ± 1.305 years), and this difference was statistically significant ($p < 0.05$). This result indicates that the longer duration of the disorder causes more severe inflammation.

RP was much more common in the progressive forms of MS, SP and PP, compared to RR form of MS ($p < 0.001$, Fisher's test Ho $p = 0.00003$) (Table 1). RP was not found in the remission phase of MS.

In the group with RP visual acuity was on average 0.79, while in the group without RP visual acuity was on average 0.90. In the whole group visual acuity on average was 0.87.

Albumin ratio values were increased in the group with RP (normal range < 5.7) and within the normal range in the group of patients without RP (Table 2), thus there was a statistically significant difference between albumin ratio values between the two groups. ($p < 0.001$) (Table 2).

Also, CSF protein level was significantly higher in the group of patients with RP compared to the group without RP ($p < 0.001$) (Table 2). In the group without RP, CSF protein levels were within the normal range (< 0.45 g/l), while in the group with RP CSF protein level was increased.

Table 1

Presence of retinal periphlebitis (RP) according to gender and multiple sclerosis (MS) type

Gender of patients and MS type	Patients (n)	
	with RP	without RP
Gender		
male	4	15
female	5	21
MS type		
remittent-relapsing form	0	28
primary and secondary-progressive form	9	8

Table 2

The average and SD values of IgG index, number of cells, intrathecal IgG synthesis (Tourtelotte), albumin ratio, CSF protein levels and types of lesions on MRI in the group of patient with and without retinal periphlebitis (RP)

Parameters	Patients	
	with RP	without RP
IgG index, $\bar{x} \pm SD$	1.388 ± 0.422	0.824 ± 0.495
Number of cells, $\bar{x} \pm SD$	3.778 ± 2.635	2.7 ± 1.4
De novo intrathecal IgG synthesis, $\bar{x} \pm SD$	26 ± 20.27	9.58 ± 13
MR lesions (n)		
old	21	1
fresh and old	15	8
Albumin ratio, $\bar{x} \pm SD$	7.55 ± 0.831	4.94 ± 2.14
CSF ptotein levels (g/L), $\bar{x} \pm SD$	0.556 ± 0.08	0.344 ± 0.125

MRI – magnetic resonance imaging; CSF – cerebrospinal fluid

The result was indicative of the active inflammatory response of the CNS.

Comparing the average values of the number of cells, it was found out that they were statistically much higher in the group of patients with retinal periphlebitis ($p < 0.01$) (Table 2).

Both parameters of intrathecal IgG synthesis, IgG index and IgG synthesis according to Tourttelotte formula, were found to be statistically higher in the group of patients with RP ($p < 0.001$) (Table 2). At the same time, intrathecal IgG synthesis was increased only in thegroup with RP, since IgG index and IgG synthesis according to Tourttelotte formula were above the normal range (0.7 and -3.3–9.9, respectively) in this group.

In regard to MRI lesions, it was found that Gd+ lesions were much more common in the group of patients with RP ($p < 0.025$, Fisher's test Ho $p = 0.012$) (Table 2).

The values of VEP`s latency were significantly higher in the group of patients with RP, in comparison to the group without RP (Table 3), at the same time the values of VEP`s amplitude was found to be significantly lower in the group of patients with RP (Table 3).

Table 3

The average and SD values of amplitude and latency of visually evoked potentials (VEP) in the group of patients with and without retinal periphlebitis (RP)

Parameters	Patients	
	with RP	without RP
VEP amplitude (μV)	3.177 ± 1.307	5.732 ± 3.704
VEP latency (μV)	148.5 ± 19.27	131.208 ± 24.01

Discussion

In our study, the occurrence of RP was 20% (9 patients). In previous studies, data varies from 5.9% to 36% [29–31, 40–42]. The differences in the reported incidence of RP in various studies are first of all due to different interpretation of changes in the retina (all changes are explained in terms of periphlebitis or only as Rucker's sign) [43]. The differences are caused by the choice of subjects, monitoring time, as well as by MS type and MS presentation – relapse or remission. Longer monitoring, as well as the more severe forms of MS give rise to much more common occurrence of RP. Due to peripheral localization of the changes and the possibility that they are not to be detected in narrow iris, it is required to perform the examination in maximal mydriasis, which on the other hand may diminish the frequency of RP in the patients with MS. There were five females and four males in the group of patients with RP. The periphlebitis was bilateral in four patients, and in five patients, it occurred only unilaterally. In the literature available to us, we found out data on both eyes being affected in patients with multiple sclerosis only in the reports on individual cases. Therefore, we cannot correlate the data with other studies. We would like to stress that if both eyes are affected, it indicates neurological progression and worse clinical prognosis of MS [5, 15]. These patients developed SP form of MS. In the group of patients with RP all the patients had either primary (four patients) or secondary (five patients) progressive form of MS, and neither had RR form of the disease. This is in accordance with certain studies affirming the correlation between the presence of these changes and clinical evidence of the MS progression [5, 15]. We consider that the differences in the study results are due to the way in which the subjects were chosen, and to the monitoring time. In the whole group of patients, RP as the initial mark of the disorder occurred in one case, first in one eye then in the other (11.5%). In relation to the very beginning of disorder, periphlebitis occurred on average after 4.22 years, which was in accordance with the data from the literature [11, 29]. Longer duration of the disorder causes the more severe extent of inflammation and evolution of RP.

Minor differences in the visual acuity between the group of patients with and without RP are mainly caused by peripheral localization of retinal changes [19, 42, 44]. The RP was an initial symptom of multiple sclerosis in one patient (2.22%). These data are in accordance with the data from the literature [29, 45–47]. Our research detected statistically higher values of albumin ratio in the group of patients with RP, which is in accordance with the data from the literature [48, 49]. This finding indicates the blood-brain barrier impairment, i.e. active inflammation within the CNS. Moreover, in the same group MRI Gd+ were much more common ($p < 0.001$) compared to the group without RP (Table 2). Both parameters indicated a blood-brain barrier damage in the group of MS patients with RP, while in non RP group blood-brain barrier was intact. In previous studies, no relation was found between the damaged blood-brain barrier and presence of RP [8, 32]. We also found statistically increased values of IgG index and *de novo* intrathecal IgG synthesis (Tourtellote) in the group of patients with RP in comparison to the group of patients without RP. The acquired result indicates an increased immunologic activity within the CNS in MS patients with RP. These results are in accordance with the results in the literature [48, 50].

Within our research we determined the parameters of VEP. In the group of patients with RP, the low amplitude and prolonged latency were registered more commonly, in contrast to the group without RP. During the research, we paid attention to pathologically lowered amplitude, which indicated acute lesion, i.e. severe inflammatory reaction resulting in the lower amplitude and significant prolongation of latency in patients with RP. These results are in accordance with the results in the literature [1, 39, 51], although there are studies reporting differently. In our opinion, the differences in results are due to the selection of patients, since those studies included mainly outpatients with milder form of MS.

Conclusion

Our study suggests that the presentation of RP is more typical in MS patients with signs of blood-brain barrier impairment. In regard to this, RP might be considered a reliable indicator of MS activity. By monitoring RP presence and evolution, it might be possible to monitor the disease activity and treatment effects, without applying other, more expensive methods such as MRI.

R E F E R E N C E S

1. *Fuhr P, Borggrefe-Chappuis A, Schindler C, Kappos L.* Visual and motor evoked potentials in the course of multiple sclerosis. Brain 2001; 124(Pt 11): 2162–8.
2. *Simka M.* Blood brain barrier compromise with endothelial inflammation may lead to autoimmune loss of myelin during multiple sclerosis. Curr Neurovasc Res 2009; 6(2): 132–9.
3. *Duker J, Weiter JJ.* Ocular circulation. In: *Tasman W, Jaeger EA,* editors. Duane's foundations of clinical ophthalmology. New York: JB Lippincott; 1991. p. 1–34.
4. *Alm A.* Ocular circulation. In: *Hart WM*, editor. Adler's physiology of the eye: clinical application. St Louis: Mosby-Year Book; 1992. p. 198–227.
5. *Engell T, Sellebjerg F, Jensen C.* Changes in the retinal veins in acute optic neuritis. Acta Neurol Scand 1999; 100(2): 81–3.
6. *Stanford M, Mathew R.* Patterns of Retinal Vascular Involvement in the Diagnosis of Retinal Vasculitis. In: *Pleyer U, Forrester JV,* editors. Uveitis and Immunological Disorders Progress III. Berlin: Springer; 2008. p. 89.
7. *Raine C, McFarland HF, Hohlfeld R.* Multiple Sclerosis – a comprehensive text. Philadelpia: Saunders Elsevier; 2008. p. 1–9.
8. *Birch MK, Barbosa S, Blumhardt LD, O Brien C, Harding SP.* Retinal venous sheathing and the blood-retinal barrier in multiple sclerosis. Arch Ophthalmol 1996; 114(1): 34–9.
9. *Abu El-Asrar AM, Herbort CP, Tabbara KF.* Differential diagnosis of retinal vasculitis. Middle East Afr J Ophthalmol 2009; 16(4): 202–18.
10. *Yanoff M, Sassani JW.* Ocular Pathology. 6th ed. Edinburgh: Mosby Elsevier; 2009.
11. *Patte M, Rouher FN, Vernay D, Delaire JC, Bacin F.* Proliferative retinal vasculitis and multiple sclerosis: a case report. J Fr Ophtalmol 2003; 26(4): 381–5. (French)
12. *Valentincic NV, Kraut A, Rothova A.* Vitreous hemorrhage in multiple sclerosis-associated uveitis. Ocul Immunol Inflamm 2007; 15(1): 19–25.
13. *Yilmaz T, Yildirim S, Aydemir O, Çelebi S, Kükner S.* Retinal periphlebitls and uveitis in a patient with multipl sclerosis. J Retina Vitreous 2003; 11: 41–5.
14. *Matsuo T, Takabatake R.* Multiple sclerosis-like disease secondary to alpha interferon. Ocul Immunol Inflamm 2002; 10(4): 299–304.
15. *Spraul CW, Lang GE.* Periphlebitis retinae, uveitis and cystoid maculopathy in a patient with multiple sclerosis. Klin Monatsbl Augenheilkd 1999; 215(6): 373–5.
16. *Hochwarter A, Prainer C, Binder S, Stolba U.* Vitreoretinal traction syndrome in multiple sclerosis. Ophthalmologe 2004; 101(2): 153–7. (German)
17. *Burkholder BM, Osborne B, Loguidice MJ, Bisker E, Frohman TC, Conger A*, et al. Macular volume determined by optical coherence tomography as a measure of neuronal loss in multiple sclerosis. Arch Neurol 2009; 66(11): 1366–72.
18. *Narayana KM, Agrawal R, Biswas J, Arjundas D.* Recurrent anterior uveitis and healed retinal vasculitis associated with multiple sclerosis. Indian J Ophthalmol 2003; 51(1): 77–9.
19. *Zein G, Berta A, Foster CS.* Multiple sclerosis-associated uveitis. Ocul Immunol Inflamm 2004; 12(2): 137–42.
20. *Kadayifçilar S, Gedik S, Eldem B, Balaban H, Kansu T.* Panuveitis associated with multiple sclerosis complicated by cerebral venous thrombosis. Ocul Immunol Inflamm 2004; 12(2): 153–7.
21. *Inoue K, Numaga J, Joko S, Izumi S, Kato S, Kawashima H,* et al. A case of multiple sclerosis with granulomatous uveitis in Japan-use of the antilipoarabinomannan (LAM)-B test in differential diagnosis. Am J Ophthalmol 2001; 131(4):524-6.
22. *Engell T, Jensen OA, Klinken L.* Periphlebitis retinae in multiple sclerosis. A histopathological study of two cases. Acta Ophthalmol (Copenh) 1985; 63(1): 83–8.
23. *Lucarelli MJ, Pepose JS, Arnold AC, Foos RY.* Immunopathologic features of retinal lesions in multiple sclerosis. Ophthalmology 1991; 98(11): 1652–6.
24. *Engell T, Hvidberg A.* Recurrence of periphlebitis retinae in multiple sclerosis. Acta Ophthalmol (Copenh) 1985; 63(1): 80–2.
25. *Amalric P.* Structure, functions and exploration of the retina. Rev Prat 1996; 46(14): 1709–13. (French)
26. *Gass JD.* Stereoscopic atlas of macular diseases diagnosis and treatment. 4th ed. St. Louis: Mosby-Year Book; 1997. p. 494–512.
27. *Soubrane G, Coscas G.* Age-related macular degeneration. Rev Prat 1996; 46(14): 1722–9. (French)
28. *Kerrison JB, Flynn T, Green WR.* Retinal pathologic changes in multiple sclerosis. Retina 1994; 14(5): 445–51.
29. *Quentin CD, Altenhoff M, Schipper HI, Vogel M.* Retinal periphlebitis and multiple sclerosis. Incidence, cerebrospinal fluid and results of visual evoked potentials. Fortschr Ophthalmol. 1990; 87(4): 359–61. (German)
30. *Río J, Colín A, Salvador F, Tintoré M, Viguera ML, Montalbán J,* et al. Retinal periphlebitis in multiple sclerosis. A prospective study. Neurologia 1993; 8(8): 252–5. (Spanish)
31. *Tola MR, Granieri E, Casetta I, Monari P, Scorrano R, Mazzeo V,* et al. Retinal periphlebitis in multiple sclerosis: a marker of disease activity? Eur Neurol 1993; 33(2): 93–6.
32. *Schmidt S, Wessels L, Augustin A, Klockgether T.* Patients with Multiple Sclerosis and concomitant uveitis/periphlebitis retinae are not distinct from those without intraocular inflammation. J Neurol Sci 2001; 187(1–2): 49–53.
33. *Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC,* et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983; 13(3): 227–31.
34. *Kurtzke JF.* Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983; 33(11): 1444–52.
35. *Tourtellotte WW, Potvin AR, Ma IB, Baum Hefner RW, Walsh MJ, Dickstein P*, et al. Measurement of intra-blood-brain barrier IgG synthesis in multiple sclerosis. A review. In: *Kuroiwa J, Kurland LT*, editors. Multiple sclerosis East and West. Basel: Karger; 1982. p. 235–48.
36. *Fazekas F, Offenbacher H, Fuchs S, Schmidt R, Niederkorn K, Horner S*, et al. Criteria for an increased specificity of MRI interpretation in elderly subjects with suspected multiple sclerosis. Neurology 1988; 38(12): 1822–5.
37. *Miller DH, Albert PS, Barkhof F, Francis G, Frank JA, Hodgkinson S*, et al. Guidelines for the use of magnetic resonance techniques in monitoring the treatment of multiple sclerosis. US National MS Society Task Force. Ann Neurol 1996; 39(1): 6–16.
38. *Jovičić A.* Elektrodiagnostics in neurology. Belgrade: Studio MZ; 1995. (Serbian)
39. *Fischer C, André-Obadia N, Mauguière F.* Diagnostic criteria of multiple sclerosis: electrophysiological criteria. Rev Neurol (Paris) 2001; 157(8-9 Pt 2): 974–80. (French)
40. *Gupta V, Balsan R, Gupta A.* Vitritis in Indian population. J Bom Ophthalmol 2004; 13(2): 43–50.
41. *Sibinelli MAMF, Cohen R, Ramalho AM, Tilbery CP, Lake JC.* Ocular manifestations in patients with multiple sclerosis in São Paulo. Arq Bras Oftal 2000; 63(4): 287–91. (Portuguese)
42. *Masoud MT.* Diagnosis and treatment of multiple sclerosis: ocular manifestations of multiple sclerosis. BMJ 2006; 332(7542): 668.

43. *Hörnsten G.* The relation of retinal periphlebitis to multiple sclerosis and other neurological disorders. Acta Neurol Scand; 1971; 47(4): 413–25.
44. *Shams PN, Plant GT.* Optic neuritis: a review. Int MS J 2009; 16(3): 82–9.
45. *Barnes D, McDonald WI.* The ocular manifestations of multiple sclerosis. Abnormalities of eye movements. J Neurol Neurosurg Psychiatry 1992; 55(10): 863–8.
46. *Friedman SM.* Retinal vasculitis as the initial presentation of multiple sclerosis. Retina 2005; 25(2): 218–9.
47. *Chen L, Gordon LK.* Ocular manifestations of multiple sclerosis. Curr Opin Ophthalmol 2005; 16(5): 315–20.
48. *Engell T.* Neurological disease activity in multiple sclerosis patients with periphlebitis retinae. Acta Neurol Scand 1986; 73(2): 168–72.
49. *Annunziata P, D'Ettorre M, Menchini U, Moretti L, Guazzi GC.* Frequency of blood-retina and blood-brain barrier changes in multiple sclerosis. Ital J Neurol Sci 1988; 9(4): 345–9.
50. *Lebrun C, Bensa C, Debouverie M, Wiertlevski S, Brassat D, de Seze J,* et al. Association between clinical conversion to multiple sclerosis in radiologically isolated syndrome and magnetic resonance imaging, cerebrospinal fluid, and visual evoked potential: follow-up of 70 patients. Arch Neurol 2009; 66(7): 841–6.
51. *Hood DC, Chen JY, Yang EB, Rodarte C, Wenick AS, Grippo TM,* et l. The role of the multifocal visual evoked potential (mfVEP) latency in understanding optic nerve and retinal diseases. Trans Am Ophthalmol Soc 2006; 104: 71–7.

Received on December 16, 2009.
Revised on March 23, 2010.
Accepted on March 26, 2010.

ORIGINALNI ČLANAK

UDC: 616-036.22::616.31-053.2(497.1)
DOI: 10.2298/VSP1107550D

Stanje oralnog zdravlja kod dece uzrasta od 12 godina u Crnoj Gori

The state of oral health in children at the age of 12 in Montenegro

Mirjana Djuričković*, Mirjana Ivanović†

***Medicinski fakultet,** Studijski program stomatologije, **Podgorica, Crna Gora;**
†Stomatološki fakultet, Klinika za dečju i preventivnu stomatologiju, **Beograd, Srbija**

Apstrakt

Uvod/Cilj. Oralno zdravlje je veoma važno za funkcionisanje i kvalitet života ljudi. Cilj ovog rada bio je utvrđivanje rasprostranjenosti karijesa na stalnim zubima, stanja parodoncijuma i stanja oralne higijene kod dece uzrasta 12 godina u Crnoj Gori. **Metode.** Istraživanje je sprovedeno tokom 2006, a obuhvatilo je 455 učenika osnovnih škola, oba pola, uzrasta 12 godina u severnoj, srednjoj i južnoj regiji Crne Gore. Parametri korišćeni za procenu stanja oralnog zdravlja bili su indeksi prosečnog broja karijesnih, izvađenih i zuba plombiranih zbog karijesa (*Mean number of decayed, missing, and filled teeth due to caries* – DMFT), *Significant Caries Index* (SiC) i *Community Periodontal Index of Treatment Needs* (CPITN), a za procenu stanja oralne higijene indeks mekih naslaga prema Green-Vermillion-u i indeks zubnog kamenca prema Green-u. Jedan stomatolog klinički je pregledao sve ispitanike u skladu sa metodologijom i kriterijumima Svetske Zdravstvene Organizacije (SZO). Sva izabrana deca iz uzorka pregledana su standardnim stomatološkim dijagnostičkim sredstvima (stomatološko ogledalce, stomatološka sonda 0,4 mm i standardna parodontalna CPITN sonda) pri veštačkom osvetljenju na suvim zubima, na stomatološkoj stolici. **Rezultati.** Prosečna vrednost DMFT indeksa za 12-godišnjake iz Crne Gore iznosila je 3,43. Karijes je imalo prosečno 88,35% pregledane dece. Indeks SiC iznosio je 6,35. Među ispitanom decom 11,9% je imalo najmanje jedan zub sa prisutnim zalivačem fisura. Zdravi parodoncijum imalo je 64% 12-godišnjaka. Prosečna vrednost indeksa mekih naslaga iznosila je 1,086, a indeksa čvrstih naslaga 0,6508. **Zaključak.** Nakon ovih epidemioloških istraživanja zaključujemo da stanje oralnog zdravlja dece ovog uzrasta u Crnoj Gori nije zadovoljavajuće. Shodno tome, treba naglasiti značaj savramenih preventivnih mera i programa, implementirati ih kroz sistem primarne zdravstvene zaštite i intenzivno raditi na promociji oralnog zdravlja.

Ključne reči:
deca; usta, zdravlje; usta, higijena; zub, karijes; lečenje; periodontalni indeks; dijagnoza.

Abstract

Background/Aim. Oral health is very important for the function and the quality of human life. The aim of this study was to determine the spread of caries on the permanent teeth, the state of health of the periodontium and the state of oral hygiene in the children at the age of 12 in Montenegro. **Methods.** The research was carried out within 2006 and included 455 primary school pupils of both sex, the age of 12 in the northern, midlle and southern area of Montenegro. The parameters used to estimate oral health condition were: mean number of decayed, missing, and filled teeth due to caries (DMFT), Significant Caries Index (SiC), Community Periodontal Index of Treatment Needs (CPITN), presence of sealants, and to estimate oral hygiene condition: Debris Index (Green-Vermillion) and Calculus Index (Green). A dental team clinically examined all the subjects in line with World Health Organization (WHO) methodology and criteria. All chosen children from the sample were checked by the standard dental diagnostic equipment (plane dental mirror, dental, standard CPITN periodontal probe) under the artificial light on the dry teeth, on the dental chair. **Results.** The average value of Index DMFT at 12-year-old in Montenegro was 3.43. On average, 88.35% of the examined children had dental caries. The SiC Index was 6.35. Among the examined children, 11.9% had at least one tooth with a fissure sealant. The healty periodontium had 64% of the 12-year-old children. The average value of Debris Index was 1.086, and the average value of Calculus Index was 0.6508. **Conclusion.** Oral health condition in children at the age of 12 in Montenegro does not satisfy. Thus the importance of the modern preventive measures and programmes should be emphasized and applied through the system of primary oral protection and intensively promote oral health.

Key words:
child; oral health; oral hygiene; dental caries; therapeutics; periodontal index; diagnosis.

Correspondence to: Mirjana Djuričković, Medicinski fakultet, Studijski program stomatologije, Ljubotinjskih junaka br. 23, 81 000 Podgorica, Crna Gora. Tel.: +382 690 778 09. E-mail: miradjurickovic@gmail.com

Uvod

Oboljenja usta i zuba sasvim opravdano smatraju se ozbiljnim zdravstvenim problemom zbog njihove velike prevalencije, kao i komplikacija koje izazivaju. Karijes predstavlja jedno od najčešćih oralnih oboljenja svih starosnih grupa.

Brojna epidemiološka istraživanja, sprovedena u dečijoj populaciji, ukazuju na veliku razliku u rasprostranjenosti karijesa među pojedinim zemljama. Rezultati ovih studija ukazuju na činjenicu da je tiha epidemija karijesa poslednjih godina gotovo zaustavljena u zemljama zapadne i severne Evrope i SAD [1–4]. Sprovođenje sistematskih školskih preventivnih programa, programa zdravstvenog vaspitanja, masovna i kontinuirana primena fluorida, poboljšana oralna higijena, promena načina ishrane i uslova života najčešće su pripisivani razlozi ovakvog unapređenja oralnog zdravlja [5]. Međutim, u zemljama istočne i centralne Evrope karijes i dalje predstavlja ozbiljan zdravstveni i socijalni problem [1, 5].

Na području Crne Gore ovaj problem je takođe izražen, a posebno je značajno istaći da do sada nisu bili dostupni ni tačni podaci o oralnom zdravlju 12-godišnjaka, kao standardizovanoj skupini za praćenje oralnog zdravlja, prema preporukama Svetske zdravstvene organizacije (SZO). Epidemiološka istraživanja ovog tipa nisu rađena u Crnoj Gori poslednjih 25 godina, pa do danas nema preciznih podataka o stanju zdravlja usta i zuba dece Crne Gore. Retka ranija istraživanja vezana za oralno zdravlje dece u Republici Crnoj Gori (RCG) bila su sporadična i necelovita, a obuhvatala su pojedine grupacije različitih uzrasta u nekim mestima RCG, ali i ovakva istraživanja ukazivala su na veliku rasprostranjenost i aktivitet oralne patologije.

U zdravstvenom sistemu Crne Gore trenutno je akcenat na kurativnom pristupu, a ne na preventivnim merama, tako da je očigledan nedostatak prevencije oralnih bolesti u primarnim zdravstvenim ustanovama, a, takođe, nedostaje i promocija oralnog zdravlja. Crna Gora je područje sa niskim udelom fluora u vodi za piće (0,05–0,2 mg/L). Zato se preporučuje upotreba zubnih pasta sa fluoridima, dostupnih u maloprodaji.

Cilj ovog rada bio je da se utvrdi stanje oralnog zdravlja dece uzrasta 12 godina u Crnoj Gori.

Metode

U izboru uzorka za ovo istraživanje vodilo se računa da izabrana deca reprezentuju u svim oblastima studije svu decu Crne Gore uzrasta 12 godina. Ovi uslovi ispunjeni su izborom 5% uzorka dece po regionima RCG (severni, srednji, južni). Uzorkom je obuhvaćeno 455 dece oba pola uzrasta 12 godina pri čemu je broj dece muškog i ženskog pola bio približno jednak. Broj ispitanika u ovim regionima, odnosno opštinama sa predgrađima, zavisio je od broja stanovnika – dece u njima. U opštinama severnog regiona i njihovih predgrađa (Berane, Bijelo Polje, Kolašin) pregledano je 159 dece, u opštinama srednjeg regiona sa predgrađima (Podgorica, Nikšić, Cetinje, Danilovgrad) 195, a u opštinama južnog regiona (Bar, Budva, Kotor) 101 dete.

Spisak svih osnovnih škola dobijen je od Ministarstva prosvete, a metodom slučajnog uzorka odabrano je 15 škola. Nakon odobrenja Etičkog komiteta i dobijanja saglasnosti direktora osnovnih škola, započet je pregled dece, prema unapred dogovorenim datumima. Ispitanici koji su učestvovali u ovoj studiji izabrani su metodom slučajnog uzorka. Sva izabrana deca iz uzorka pregledana su standardnim stomatološkim dijagnostičkim sredstvima (stomatološko ogledalce, stomatološka sonda, standardna parodontalna CPITN (*Community Periodontal Index for Treatment Needs*) sonda pri veštačkom osvetljenju, na suvim zubima na stomatološkoj stolici, u školskoj stomatološkoj ambulanti [6]. U istraživanje su bila uključena samo deca rođena 1994, a ova studija realizovana je tokom 2006.

Jedna stomatološka ekipa je posetila osnovne škole i klinički pregledala sve ispitanike prema metodologiji i kriterijumima SZO. Parametri korišćeni za procenu stanja oralnog zdravlja bili su indeksi: *Mean number of decayed, missing or filled teeth due to caries* (DMFT), odnsono karijes, ekstrakcija, plomba (KEP) zuba, *Significant Caries Index* (SiC), CPITN, a za procenu stanja oralne higijene: indeks mekih naslaga prema Green-Vermillion-u (Indeks debris) kojim se utvrđuje odsustvo, odnosno prisustvo, količina i rasprostranjenost dentalnog plaka i ostalih mekih naslaga na zubima kao i indeks zubnog kamenca prema Green-u (Indeks kalkulus) u kojim se utvrđuje odsustvo, odnosno prisustvo zubnog kamenca i subgingivalnih konkremenata na zubima [6].

Karijesni status registrovan je pomoću Klein-Palmerovog sistema DMF (D-*Decayed*, M-*Missing*, F-*Filled*) koji je kod nas preveden u KEP (K-karijes, E-ekstrahovan zub, P-plombiran zub). Jasno vidljive lezije sa formiranim kavitetom na površini zuba registrovane su kao dentalni karijes, dok su promene u transparenciji i početne demineralizacije gleđi sa intaktnom površinom, bez kavitacije, registrovani kao zdravi zubi [6].

Indeks SiC, preveden kao Indeks značajnog karijesa, predstavlja gornju trećinu frekvencijske raspodele DMFT (KEP). Uveden je sa ciljem da ukaže na osobe sa najvišim vrednostima karijesa u svakoj populaciji. Ovaj indeks se koristi kao dopuna srednjim vrednostima DMFT (KEP), i daje pravu sliku pacijenata sa rizikom od karijesa [6]. Dobija se na sledeći način: sva pregledana dece sortiraju se prema vrednostima KEP; zatim jedna trećina pregledane dece sa najvišim vrednostima KEP selektuje se i dobijeni broj predstavlja podskup SiC, te tako dobijeni rezultat DMFT (KEP) za ovaj podskup predstavlja vrednost SiC [6].

Procena stanja parodoncijuma zabeležena je indeksom CPITN, prema preporukama SZO za ispitanike mlađe od 15 godina. Kod dece ovog uzrasta ne preporučuje se merenje dubine sulkusa, tj. džepova, pa se stanje parodoncijuma određuje na osnovu kliničkog stanja gingive i eventualnog prisustva čvrstih naslaga na zubima [6].

Kliničke preglede u školama obavljao je jedan stomatolog obučen da se služi gore navedenim indeksima. U testiranju pouzdanosti istraživača primenjivana je statistika *kappa*. *Kappa* vrednosti procenjene nakon ponovnog pregleda za intrakonzistenciju terenskog istraživača, iznosile su 0,92.

Rezultati istraživanja analizirani su uz pomoć *t*-testa, χ^2 testa, a vrednosti $p < 0,05$ smatrane su statistički značajnim.

Rezultati

Zastupljenost ispitivanih dvanaestogodišnjaka prema polu, regionu i tipu naselja u kom su bili nastanjeni prikazana je u tabeli 1.

Tabela 1

Distribucija 12-godišnjaka u Crnoj Gori prema polu, regionima i tipu naselja

Pol	Region	Tip naselja		
		grad	ostalo	svega
		n (%)	n (%)	n (%)
Muški	Severni	46 (30,9)* (56,1)†	36 (40,5)* (43,9)†	82 (34,5)* (100,0)†
	Srednji	70 (47,0)* (66,7)†	35 (39,3)* (33,3)†	105 (44,1)* (100,0)†
	Južni	33 (22,1)* (64,7)†	28 (20,2)* (35,3)†	51 (21,4)* (100,0)†
Ukupno		149 (100,00)* (62,6)†	89 (100,00)* (37,4)†	238 (100,0)* (100,0)†
Ženski	Severni	45 (32,4)* (58,4)†	32 (41,0)* (41,6) †	77 (35,5)* (100,0)†
	Srednji	61 (43,9)* (67,8)†	29 (37,2)* (32,2)†	90 (41,5)* (100,0)†
	Južni	33 (23,7)* (66,0)†	17 (21,8)* (34,0)†	50 (23,0)* (100,0)†
Ukupno		139 (100,0)* (64,1)†	78 (100,0)* (35,9)†	217 (100,0)* (100,0)†
Oba pola	Severni	91 (31,6)* (57,2)†	68 (40,7)* (42,8)†	159 (57,2)* (100,0)†
	Srednji	131 (45,5)* (67,2)†	64 (38,3)* (32,8)†	195 (42,9)* (100,0)†
	Južni	66 (22,9)* (65,3)†	35 (21,0)* (34,7)†	101 (22,2)* (100,0)†
Ukupno		288 (100,0)* (63,3)†	167 (100,0)* (36,7)†	455 (100,0)* (100,0)†

***procentualna zastupljenost po vertikali; †procentualna zastupljenost po horizontali**

Kod ove dece posmatrano je stanje stalnih zuba kroz karijesne indekse koji se odnose na opštu frekvenciju karijesa (Kio) (DMFT *persons*), Kip (DMFT) i strukturu KEP (struktura DMFT).

Procenat dece sa obolelim stalnim zubima za uzrast 12 godina ukupno za RCG iznosio je 88,35%, dok je 11,41% dece ove dobi imalo zdrave sve zube. Statistički značajnih razlika u vrednostima ovog indeksa karijesa nije bilo u odnosu na pol i region (tabela 2). Najniži procenat dece sa obolelim zubima zabeležen je u južnom regionu (86,14%), a najviši u severnom (89,94%). U sva tri regiona ustanovljen je viši procenat devojčica za zdravim stalnim zubima (tabela 2).

ne. Deca gradskih područja imala su značajno niže vrednosti ovog indeksa u odnosu na svoje vršnjake iz vangradskih područja (2,99 *vs* 4,19; $t = 16,782$, $p < 0,001$). Takođe, značajno niže vrednosti Kip-a ($t = 5,266$; $p < 0,001$) zabeležene su kod dečaka vangradskih područja (3,02), u odnosu na dečake ostalih područja (4,55), (tabela 2). Devojčice prigradskih naselja imale su više vrednosti ovog indeksa (3,77), od devojčica iz grada (2,95), što je ocenjeno kao statistički veoma značajna razlika ($t = 13,003$; $p < 0,001$), (tabela 2).

U strukturi DMFT (KEP) dominirao je nesanirani karijes (49,13%), zatim slede zubi sa ispunima (42,27%) i manji postotak ekstrahiranih zuba (8,60%). Strukturni odnos karijes : ekstrakcija : plombe u gradskom području bio je 44,19% : 8,60% : 47,21%, a u ostalom 55,22% : 8,58% : 36,20%, što je na nivou verovatnoće ocenjeno kao visoko statistički značajno ($x^2 = 20,624$; $p < 0,001$; $df = 2$). Od ukupno 1 559 karioznih, ekstrahovanih ili plombiranih zuba

Tabela 2

Opšta zastupljenost karijesa (Kio) prosečan broj karijesnih zuba po osobi (Kip) i prosečna vrednost indeksa debris kod dece u Crnoj Gori uzrasta od 12 godina u odnosu na pol, region i tip naselja

Region	Parametar	Grad	Ostalo	Svega
		M / Ž / M+Ž	M / Ž / M+Ž	M / Ž / M+Ž
Severni	Kio	89,13 / 86,66 / 87,91	94,44 / 90,62 / 92,65	91,46 / 88,31 / 89,94
Srednji		91,42 / 91,96 / 87,02	88,57 / 93,10 / 90,63	90,46 / 85,55 / 88,21
Južni		81,81 / 84,84 / 83,83	88,88 / 94,11 / 91,43	84,31 / 88,00 / 86,14
Ukupno		88,59 / 84,17 / 86,46	91,01 / 92,31 / 91,62	89,50 / 87,10 / 88,35
Severni	Kip	2,86 / 3,20 / 3,03	5,69 / 4,06 / 4,93	4,10 / 3,55 / 3,84
Srednji		3,34 / 2,80 / 3,31	4,11 / 4,03 / 4,08	3,61 / 3,20 / 3,42
Južni		2,54 / 2,87 / 2,71	3,11 / 2,76 / 2,94	2,74 / 2,84 / 2,79
Ukupno		3,02 / 2,95 / 2,99	4,55† / 3,77† / 4,19†	3,59 / 3,24 / 3,43
Severni	Indeks debris	0,831 / 0,664 / 0,748	1,889 / 0,998 / 1,469*	1,360* / 0,821 / 1,098
Srednji		0,822 / 1,142 / 0,939	1,583 / 1,000 / 1,333†	1,075 / 1,089 / 1,081
Južni		0,942 / 1,1,35 / 1,048	0,898 / 1,386 / 1,142	0,926 / 1,216* / 1,079
Crna Gora		0,856 / 0,992 / 0,919	1,552* / 1,10 / 1,343†	1,130 / 1,035 / 1,086

M – muški pol; Ž – ženski pol; M + Ž – oba pola; *$p < 0,05$; †$p < 0,001$

Prosečan broj obolelih zuba po jednom ispitaniku, odnosno DMFT (Kip) indeks, ukupno za svu decu uzrasta 12 godina u Crnoj Gori iznosio je 3,43, a kretao se od 2,79 u južnom regionu do 3,84 u severnom regionu (tabela 2). U odnosu na pol, vrednosti ovog indeksa bile su veoma ujednačekod 12-godišnjaka oba pola, 855 zuba je bilo kod dečaka (450 u gradu, 405 izvan grada), a 704 kod devojčica (410 u gradu, 294 izvan grada) (tabela 3). Zapažene su demografske razlike (regionske), kao i razlike u odnosu na pol (tebele 3 i 4).

Tabela 3

Zastupljenost karioznih (K), ekstrahovanih (E) i plombiranih (P) zuba kod dece u Crnog Gori uzrasta od 12 godina u odnosu na pol i tip naselja

Struktura KEP	Grad	Ostalo	Svega
	M / Ž / M+Ž	M / Ž / M+Ž	M / Ž / M+Ž
K	52,22 / 35,37 / 44,19	55,31 / 55,10 / 55,22*	53,68 / 46,61 / 49,13
E	5,56 / 11,95 / 8,60	8,15 / 9,18 / 8,58	6,78 / 10,80 / 8,60
P	42,22 / 52,68 / 47,21*	36,40 / 35,72 / 36,20	39,54 / 45,59 / 42,27
Ukupno	100 / 100 / 100	100 / 100 / 100	100 / 100 / 100
Zubi sa KEP			
n	450 / 410 / 860	405 / 294 / 699	855 / 704 / 1559
%	52,63 / 58,24 / 55,16*	47,37 / 41,76 / 44,84	100 / 100 / 100

M – muški pol; Ž – ženski pol; M + Ž – oba pola; *$p < 0,05$; †$p < 0,001$

Tabela 4

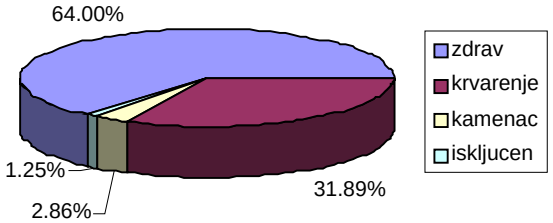
Zastupljenost karioznih (K), ekstrahovanih (E) i plombiranih (P) zuba kod dece uzrasta od 12 godina u različitim regionima Crne Gore

Region	Pol	Struktura KEP (%)		
		K	E	P
Severni	Muški	58,16	8,60	33,23
	Ženski	52,91	14,59	32,48
Srednji	Muški	46,84	7,10	46,05
	Ženski	31,59	9,02	59,37
Južni	Muški	60,71	2,85	36,42
	Ženski	50,00	7,04	42,95

Podskup SiC činilo je 152 dece. SiC indeks (gornja trećina frekvencijske raspodele DMFT, tj. Kip) za 12-godišnjake u Crnoj Gori iznosio je 6,35.

Bilo je 11,9% dece koja su imala makar jedan prisutan zalivač fisura u ustima.

Kada je reč o parodontalnom statusu kompletnog uzorka, 64% 12-godišnjaka imalo je zdrava parodontalna tkiva (slika 1).



Sl. 1 – Stanje periodoncijuma kod dece uzrasta od 12 godina iz Crne Gore prema najvišim *Community Periodontal Index* (CPI) bodovima po osobi

Ispitivanja stanja oralne higijene pokazala su da je prosečna vrednost indeksa debris za 12-godišnjake iz Crne Gore iznosila 1,086. Devojčice, ukupno za Crnu Goru (1,035), imale su niže vrednosti ovog indeksa u odnosu na dečake (1,130). Deca gradskih područja imala su niže prosečne vrednosti indeksa debris (0,919) u odnosu na decu ostalih područja (1,343), što je statistički značajno ($t = 11,640$; $p < 0,001$). Ukupno za Crnu Goru, dečaci uzrasta 12 godina iz vangradskih naselja imali su veće vrednosti ovog indeksa (1,552), od svojih vršnjaka iz gradskih sredina (0,856), što je ocenjeno kao visoko statistički značajno ($t = 10,527$; $p < 0,05$), (tabela 2). Takođe, odnos prosečnih vrednosti indeksa debris kod pregledane dece oba pola iz gradskih, u odnosu na vangradska područja, bio je u severnom regionu 0,748 : 1,469 ($t = 6,725$; $p < 0,001$), u srednjem 0,939 : 1,333 ($t = 5,521$; $p < 0,001$) i u južnom regionu 1,048 : 1,142 ($t = 1,517$; $p > 0,05$). Odnos prosečnih vrednosti indeksa debris u odnosu na pol, region i tip naselja kod dece uzrasta 12 godina u Crnoj Gori mogu se bolje sagledati u tabeli 2.

Procentualna zastupljenost dece sa prisutnim čvrstim naslagama na zubima u ovom uzrastu bila je vrlo niska i iznosila je 2,86%. Prosečna vrednost indeksa kalkulus za sve ispitanike iz uzorka bila je 0,6508 ± 0,4661.

Diskusija

Rezultati ovog istraživanja ukazuju na nešto višu rasprostranjenost karijesa kod 12-godišnjaka u Crnoj Gori (DMFT – 3,43). Ako se ove vrednosti uporede s iznosom od 6,9 dobijenim u studiji koju je sproveo Vrbić [7] 1986. u vreme bivše SFRJ (to je jedina studija u koju su deca ovog uzrasta u Crnoj Gori) jasno je da se oralno zdravlje dece ovog uzrasta poboljšalo. Jedan od razloga poboljšanja oralnog zdravlja treba tražiti i u preventivnom programu čija je implementacija započeta početkom 90-tih godina prošlog veka, a koji je kasnije prekinut zbog nedostatka finansija i ratnih dešavanja na prostorima bivše SFRJ. U svakom slučaju, teško je objasniti razloge za to, jer su preduzete mere bile ograničene uglavnom na lokalni nivo (npr. školski programi, opštinski edukativni projekti, školske promocije oralnog zdravlja, itd). Jedno od mogućih objašnjenja jeste naše korišćenje metodologije SZO u dijagnozi karijesa, prema kojoj su isključeni zubi zahvaćeni promenama koje se javljaju pre klinički prepoznatljivih gleđnih lezija ili promene slične karijesu [6].

Međutim, ako se izvrši poređenje sa sličnim epidemiološkim istraživanjima iz okruženja, ali i šireg područja, dobija se, nimalo ohrabrujuća slika.

Na osnovu dobijenih vrednosti DMFT-a, možemo zaključiti da Crna Gora pripada skupu zemalja sa rizikom po pitanju rasprostranjenosti karijesa [8].

Kad su u pitanju zemlje okruženja, prosečne vrednosti broja obolelih stalnih zuba po jednom ispitaniku kreću se u rasponu od 4,16 u Bosni i Hercegovini [9], 3,5 u Hrvatskoj, 3,0 u Makedoniji [10, 11] do 2,8 u Srbiji [12], što ukazuje na činjenicu da su rat, sankcije i nemaština, između ostalog, uticale na ovakvu sliku oralnog zdravlja kod dece na prostoru bivše SFRJ.

Upoređivanje vrednosti DMFT kod dece uzrasta od 12 godina u Crnoj Gori sa onima ustanovljenim za istu populacionu grupu, koje se kreću u rasponu od 3,24 u Meksiku, 2,4 na Filipinima, 1,8 u Sloveniji, 1,7 u Portugalu, 1,07 u Španiji, 1,66 u Izraelu, 1,1 u Švedskoj, 0,9 u Danskoj i Švajcarskoj do najnižih vrednosti od 0,65 i 0,5 u Nikaragvi i Indiji, upućuje na činjenicu da je stanje oralnog zdravlja naših 12-godišnjaka zabrinjavajuće [10–18].

Poslednjih godina posebna pažnja posvećuje se visokorizičnim pojedincima, i, s tim u vezi, vrši se analiza prosečnog DMFT indeksa trećine najviše pogođenih ispitanika. Tokom 2005. godine, vrednost prosečnog DMFT indeksa za 12-godišnjake u Švedskoj iznosio je 1,0, a indeks SiC 2,9. Godine 2004. vrednost SiC u Italiji iznosila je 3,1, a DMFT indeksa 1,1 [10, 11]. U Velikoj Britaniji za 2001. SiC indeks za 12-godišnjake iznosio je 3,2 [19]. Zabeležena vrednost ovog indeksa za mališane iz Crne Gore bila je 6,35, što je mnogo više od navedenih vrednosti, zbog čega je poželjna redukcija na manje od 3 do 2015. godine, kako bi se postiglo poboljšanje oralnog zdravlja prema zahtevima koje je postavio Bratthall [20].

Procenat ispitanika sa barem jednim prisutnim zalivačem fisura u Crnoj Gori bio je vrlo nizak (11,9%), naročito u poređenju sa Danskom, gde je kod dve trećine pregledanih 15-godišnjaka naveden bar jedan zalivač [21]. Mali procenat zalivenih fisura imala su i deca u Bosni i Hercegovini (5,7%) [9]. Ovaj nimalo ohrabrujući rezultat upućuje na prilično retku upotrebu ove značajne preventivne mere kod stomatologa Crne Gore. Zalivači fisura dokazani su kao dobra profilaktička mera u prevenciji, odnosno kontroli karijesa, pa se zbog toga moraju primenjivati.

Rezultati indeksa CPITN bili su, uglavnom, u skladu sa smernicama SZO, za tu populacionu grupu [6]. Međutim, deca iz Crne Gore lošije se kotiraju kad je u pitanju oboleli periodoncijum, u odnosu na 12-godišnjake Portugala, gdje je samo 12,5% ispitanika imalo CPI skor 1 i 2 (krvarenje i kalkulusi) [22]. Pa ipak, u odnosu na vršnjake iz okruženja (Bosna i Hercegovina [9], Kosovo i Metohija [23]), 12-godišnjaci iz Crne Gore imaju bolje stanje potpornog aparata. Mnoga stanja koja je potrebno lečiti (krvarenje – 32%, kamenac – 2,86%), prisiljavaju nas na što raniju prevenciju i dijagnozu parodontalne bolesti kako bi to postao važan deo strategije bržeg poboljšanja oralnog zdravlja. Rana dijagnoza u detinjstvu i eliminacija mogućih rizičnih faktora mogu kod odraslih preduhitriti razvoj destrukcije parodontalne kosti.

Mnogobrojni radovi ukazuju na to da je dentalni plak u korelaciji sa svim oblicima obolenja parodoncijuma. Stoga je važno objektivno notirati prisustvo i lokalizaciju dentalnog plaka na zubima svakog pacijenta [24, 25]. Prosečna vrednost indeksa debris za svu pregledanu decu uzrasta 12 godina u Crnoj Gori iznosila je 1,086, a indeksa kalkulus 0,658. Dobijeni rezultati koreliraju sa promenama na periodoncijumu, odnosno kod dece sa notiranim prisutnim mekim i čvrstim naslagama (lošom oralnom higijenom), a zapažene su zapaljenske promene sa prisutnim krvarenjem na gingivi. Zdravlje gingive i peridoncijuma je izuzetno važno kod dece, a poboljšana oralna higijena je dominantna metoda kojom se ovaj cilj postiže. Efikasno uklanjanje dentalnog plaka je od presudnog značaja za zdravlje zuba i potpornog aparata tokom čitavog života. Stoga je neophodno ova saznanja primeniti za promociju oralnog zdravlja na individualnom i društvenom nivou [24, 25].

Zaključak

Glavni razlozi za trenutno stanje oralnog zdravlja dece uzrasta 12 godina u Crnoj Gori su odsustvo populacijskih preventivnih programa i, uglavnom, kurativno usmerena stomatološka politika, siromaštvo stanovništva i nedostatak promocije oralnog zdravlja.

Potrebno je preduzeti neophodne mere za poboljšanje i unapređenje oralnog zdravlja, a to su organizovanje sistema primarne zdravstvene zaštite sa posebnim akcentom na preventivnim i profilaktičkim merama, promocija oralnog zdravlja i podizanje nivoa zdravstvene svesti, na individualnom i društvenom nivou.

L I T E R A T U R A

1. *Marthaler TM.* Changes in dental caries 1953-2003. Caries Res 2004; 38(3): 173–81.
2. *Marthaler TM, O'Mullane DM, Vrbic V.* The prevalence of dental caries in Europe 1990-1995. ORCA Saturday afternoon symposium 1995. Caries Res 1996; 30(4): 237–55.
3. *Beltrán-Aguilar ED, Barker LK, Canto MT, Dye BA, Gooch BF, Griffin SO,* et al. Surveillance for dental caries, dental sealants, tooth retention, edentulism, and enamel fluorosis-United States, 1988-1994 and 1999-2002. MMWR Surveill Summ 2005; 54(3): 1–43.
4. *Clark DC, Berkowitz J.* The influence of various fluoride exposures on the prevalence of esthetic problems resulting from dental fluorosis. J Public Health Dent 1997; 57(3): 144–9.
5. *Petersen PE.* Changing oral health profiles of children in Central and Eastern Europe–Challenges for the 21st century. IC Digest 2003; 2: 12–3.
6. *World Health Organization.* Oral Health Surveys-Basic Methods. 4th ed. Geneva: WHO; 1997.
7. *Vrbic V, Vulović M, Rajić Z, Topić B, Tatić E, Malić M,* et al. Oral health in SFR Yugoslavia in 1986. Community Dent Oral Epidemiol 1988; 16(5): 286–8.
8. *Künzel W.* Zur Konversion der epidemiologischen Zucker/Karies-Relation in Europa. Oralprophylaxe 2001; 23: 66–9.
9. *Muratbegović A, Marković N, Kobašlija S, Zukanović A.* . Oral health indices and molar incisor hypomineralization in 12 year old bosnians. Acta Stomatol Croat 2008; 42(2): 155–63. (Croatian)

10. *World Health Organization*. Global Oral Health Data Bank. Geneva: World Health Organization; 2001.
11. *World Health Organization*. Oral Health Country, Caries for 12-years – olds by Country/ Area WHO Region. Geneva: WHO; 2004.
12. *Ivanović M, Carević M, Marković D, Vulićević Z, Stevanović R, Petrović V*, et al. Protocols in dentistry. Belgrade: School of Dentristry; 2009. (Serbian)
13. *Villalobos-Rodelo JJ, Medina-Solís CE, Molina-Frechero N, Vallejos-Sánchez AA, Pontigo-Loyola AP, Espinoza-Beltrán JL*. Dental caries in schoolchildren aged 6-12 years in Navolato, Sinaloa, México: experience, prevalence, severity and treatment needs. Biomedica 2006; 26(2): 224–33. (Spanish)
14. *Yabao RN, Duante CA, Velandria FV, Lucas M, Kassu A, Nakamori M,* et al. Prevalence of dental caries and sugar consumption among 6-12-y-old schoolchildren in La Trinidad, Benguet, Philippines. Eur J Clin Nutr 2005; 59(12): 1429–38.
15. *Almerich Silla JM, Montiel Company JM*. Oral health survey of the child population in the Valencia Region of Spain (2004). Med Oral Patol Oral Cir Bucal 2006; 11(4): E369–81. (English, Spanish)
16. *Zusman SP, Ramon T, Natapov L, Kooby E*. Dental health of 12-year-olds in Israel-2002. Community Dent Health 2005; 22(3): 175–9.
17. *Herrera MS, Medina-Solis CE, Maupomé G*. Prevalence of dental caries in 6-12-year-old schoolchildren in Leon, Nicaragua. Gac Sanit 2005; 19(4): 302–6. (Spanish)
18. *David J, Wang NJ, Astrøm AN, Kuriakose S*. Dental caries and associated factors in 12-year-old schoolchildren in Thiruvananthapuram, Kerala, India. Int J Paediatr Dent 2005; 15(6): 420–8.
19. *Pitts NB, Evans DJ, Nugent ZJ, Pine CM*. The dental caries experience of 12-year-old children in England and Wales. Surveys coordinated by the British Association for the Study of Community Dentistry in 2000/2001. Community Dent Health 2002; 19(1): 46–53.
20. *Bratthall D*. Introducing the Significant Caries Index together with a proposal for a new global oral health goal for 12-year-olds. Int Dent J 2000; 50(6): 378–84.
21. *Ekstrand KR, Martignon S, Christiansen ME*. Frequency and distribution patterns of sealants among 15-year-olds in Denmark in 2003. Community Dent Health 2007; 24(1): 26–30.
22. *de Almeida CM, Petersen PE, André SJ, Toscano A*. Changing oral health status of 6- and 12-year-old schoolchildren in Portugal. Community Dent Health 2003; 20(4): 211–6.
23. *Martinović B, Milojković Z, Cvetković A, Stojanović B*. The health status of the periodontium in schoolchildren in the region of Kosovo and Metochia. Praxis Medica 2009; 37(1–2): 61–4. (Serbian)
24. *Ivanović M, Vučetić M*. Promotion of gingival and periodontal health from early childhood. Stomatološki informator 2006; 12(18):19–23. (Serbian)
25. *Vulović MD, Beloica D, Gajić M, Stevanović R, Ivanović MD, Carević MR,* et al. Preventive dentistry. Belgrade: Naučna knjiga; 2002. (Serbian)

Primljen 21. XII 2009.
Revidiran 24. II 2010.
Prihvaćen 17. III 2010.

ORIGINAL ARTICLE

UDC: 616.381-091.8-07:616.61
DOI: 10.2298/VSP1107556T

Immunohistochemical study of pathological alterations of peritoneum in patients with terminal renal insufficiency and on peritoneal dialysis

Imunohistohemijsko ispitivanje patološki izmenjenog peritoneuma kod bolesnika sa terminalnom bubrežnom insuficijencijom na peritoneumskoj dijalizi

Jasna Trbojević-Stanković*, Miljana Obradović[†], Vesna Čemerikić-Martinović[‡], Dušan Trpinac[†], Željko Laušević[§], Biljana Stojimirović[‖]

***Clinical Center Dr "Dragiša Mišović – Dedinje",** Clinic of Urology, Department of Hemodialysis, **Belgrade, Serbia; [†]University of Belgrade, School of Medicine,** Institute of Histology and Embryology, **Belgrade, Serbia; [‡]Histolab, Belgrade, Serbia; Clinical Center of Serbia, [§]**Institute for Digestive Diseases, **[‖]**Institute of Urology, Clinic of Nephrology, **Belgrade, Serbia**

Abstract

Background/Aim. During peritoneal dialysis (PD) an exchange of substances between blood and dialysate takes place through specific histological structures of peritoneum. Peritoneal double-layered serous membrane has, so far, mostly been studied with electron microscopy on experimental animals. The aim of this study was to assess integrity of peritoneal tissue in end-stage renal disease (ESRD) and PD patients using standard light microscopy and immunohistochemical methods. **Methods.** Peritoneal tissue biopsies were performed on 25 persons: 8 healthy donors during nephrectomy, 9 ESRD patients upon insertion of PD catheter, and 8 PD patients upon removal of the catheter for medical indications. The samples were fixed and prepared routinely for immunocytochemical staining by standardized streptavidin biotin AEC method using a LSAB2® HRP kit (Dako®, Denmark) for collagen IV and analyzed by light microscopy. **Results.** We observed mesothelial detachment from *lamina propria*, duplicated basement membrane and much thicker blood vessel walls in ESRD and PD patients, compared to healthy subjects. Differences in histological structure, emphasized with immunostaining, indicated pathological alterations of peritoneal tissue in the renal patients. **Conclusions.** Imunohistochemistry can be used in studying histological alterations of peritoneal tissue in ESRD and PD patients. This method may indicate possible problems in filtration and secretion processes in this tissue.

Key words:
kidney failure, chronic; peritoneal dialysis; peritoneum; pathology; immunohistochemistry; microscopy.

Apstrakt

Uvod/Cilj. Tokom peritoneumske dijalize (PD) odvija se razmena materija kroz specifične elemente histološke građe peritoneumske membrane. Ova dvolisna serozna membrana do sada je, uglavnom, ispitivana elektronskom mikroskopijom na eksperimentalnim životinjama. Cilj našeg rada bio je da procenimo integritet peritoneumske membrane kod bolesnika sa terminalnom insuficijencijom bubrega (TIB) i bolesnika na PD primenom standardne svetlosne mikroskopije i imunohistohemijskih bojenja. **Metode.** Uzorci tkiva peritoneuma uzeti su od 25 osoba: osam zdravih donora bubrega, devet bolesnika sa TIB prilikom plasiranja katetera za započinjanje PD i osam bolesnika na PD prilikom uklanjanja katetera iz medicinskih razloga. Uzorci su fiksirani i rutinski pripremljeni za imunohistohemijsko bojenje standardizovanom streptavidion/biotin AEC metodom primenom LSAB2® HRP kompleta (Dako®, Denmark) za bojenje kolagena IV i analizirani svetlosnom mikroskopijom. **Rezultati.** Uočeno je odvajanje mezotela peritoneuma od lamine proprije, duplikacija bazalne membrane mezotela i značajno zadebljanje zidova krvnih sudova kod bolesnika sa TIB i na PD u odnosu na zdrave osobe. Ove promene histološke građe peritoneuma, jasno obeležene imunohistohemijskim bojenjem, ukazuju na patološke promene tkiva peritoneuma kod bubrežnih bolesnika. **Zaključak.** Imunohistohemijske metode mogu se koristiti za ispitivanje promena strukture tkiva peritoneuma kod bolesnika sa TIB i na PD. Ova metoda može pomoći u identifikovanju mogućih uzroka filtracionih i sekrecionih procesa u ovom tkivu koji utiču na efikasnost PD.

Ključne reči:
bubreg, hronična insuficijencija; dijaliza, peritoneumska; peritoneum; patologija; imunohistohemija; mikroskopija.

Correspondence to: Jasna Trbojević-Stanković, Clinical Center Dr "Dragiša Mišović – Dedinje", Department of Hemodialysis, Heroja Milana Tepića 1, 11 000 Belgrade, Seriba. Phone: + 381 3630 600, ext. 572. E-mail: jasna.ts@neobee.net

Introduction

Peritoneal membrane is involved in filtration of substances from peritoneal fluid and in the exchange of substances between blood and dialysis fluid within the peritoneal cavity during peritoneal dialysis (PD). The following histological structures of the peritoneum which form the peritoneal membrane are involved in these processes: stagnant fluid layer within peritoneal capillaries, capillary endothelium, capillary basement membrane, interstitium, mesothelium and stagnant fluid film on mesothelial surface within the peritoneal cavity. Peritoneal mesothelium possesses distinctive regenerative ability. Normal mytotic activity of rat mesothelial cells is 1% daily, and may rise up to 19% in peritonitis [1].

Standard dialysis solutions are not biocompatible with the peritoneal membrane. They contain glucose in non-physiological concentration, serving as osmotic substance, and lactates, which maintain dialysate's low pH. Both these substances exhibit negative effects on peritoneal tissue. Furthermore, during sterilization and preservation of the solution, glucose degradation products and advanced glucose degradation products are formed, which adversely affect peritoneal structure. High glucose concentration causes non-enzymatic glycosilation of tissue proteins. This may account for development of diabetiform alterations: loss of mesothelial layer, thickening of submesothelium due to increased deposition of collagen and hialuron in interstitium, interstitial fibrosis, thickening of mesothelial basement membrane and endothelial basement membrane of small peritoneal blood vessels accompanied by neoangiogenesis [2]. Structural changes affect quality of dialysis, as they increase velocity of low molecular mass solutes, increase peritoneal microvascular surface and ultrafiltration.

It is still difficult to study effects of dialysate on the peritoneal membrane in humans since tissue samples can be obtained solely when placing or removing a peritoneal catheter.

Transmission and scanning electron microscopy provided detailed description of peritoneal structure and better understanding of physiology of mesothelial cells and the peritoneal membrane as a whole, as well as pathophysiological changes in ESRD and PD patients [2–5]. Most studies were conducted on experimental animals, but some data were obtained from human biopsy material as well [6–9] .

The aim of this study was to present application of immunohistochemical methods with basic microscopic techniques (light microscopy) in studying integrity of the peritoneal membrane in the ESRD and PD patients. These data may be important in estimating efficiency of peritoneal membrane in performing filtration and secretion processes during PD.

Methods

Tissue samples

Histological characteristics of the peritoneum were studied on samples of parietal peritoneum obtained from 25 persons upon abdominal surgery. The study was approved by the local institution review board and all the subjects gave informed written consent for participation in the study according to the Helsinki Declaration. The control group included 8 healthy kidney donors, 2 males and 6 females, mean age 52.5 ± 5.2 years, with no history of previous pathological abdominal conditions or surgery. Peritoneal tissue samples were obtained upon donor nephrectomies. The ESRD group included 9 patients, 6 males and 3 females, mean age 54.2 ± 5.8 years. Three patients had endemic nephropathy, 3 had polycystic kidney disease, 2 had glomerulonephritis, and 1 had unknown kidney disease. They all underwent biopsies at the time of insertion of a PD catheter, prior to the commencement of PD. The PD group included 8 patients, 6 males and 2 females, mean age 54.7 ± 13.6 years. Basic kidney disease was glomerulonephritis in 2 of these patients, 2 had endemic nephropathy, 2 had policystic kidney disease and 2 had unknown kidney disease. Biopsies were taken upon catheter repositioning or catheter replacement due to PD related problems.

Peritoneal tissue is extremely fragile and susceptible to mechanical irritation and factors of external environment. Collection of samples was therefore perfomed strictly obliging the guidelines from the literature [10, 11].

Section preparation and immunohistochemical staining

The tissue was fixed for 24 hours in 10% formaldehyde with 0.1M Sorensen's phosphate buffer pH 7.4, dehydrated in 96% ethanol, then routinely processed for embedding in paraplast. Immunohistochemical staining was performed by the standard streptavidin biotin 9-amino-ethyl carbasol (AEC) method. Collagen IV fibers were visualised using a LSAB2® HRP kit (Dako®, Denmark) [12]. This method is based on the specific binding of primary antibody to tissue antigenes previously incubated with 3% hydrogen in order to inhibit endogenous peroxidase. After 90 minutes incubation with primary antibody for collagen IV, samples were incubated with secondary antibody bound to biotin, and then with streptavidin bound horseradish peroxidase (HRP). This complex: primary – secondary antibody – biotin – streptavidin – enzyme, was then stained with AEC chromogen, resulting in redish-brown product representing positive immunoreaction. Reaction was interrupted by rinsing with distillited water. Samples were then contrasted with Mayer hematoxylin and examined by light microscopy (Opton Photomicroscope III, original magnification 300×) [13].

Morphometric analysis

Outer and lumen diameters of peritoneal blood vessels were determined on vessels transversal sections with 3.1 Soft Imaging System GMbH, Munster, Germany, by direct measuring on the image projected from the microscope on computer screen, using a digital camera (Olympus C3030). Peritoneal blood vessels wall thickness was calculated as difference between outer and lumen diameter. The results were analyzed with ANOVA and Tuckey HSD tests (STATISTICA 5.0, StatSoft, Inc. Tulsa OK); significance was considered at $p < 0.05$.

Results

We used immunohistochemical staining to visualize peritoneal structures which were particularly exposed to pathological conditions and therefore suffered alterations: mesothelial basement membrane and blood vessels' walls. Pathological changes also affect interstitial collagen fibers, especially in the process of peritoneal fibrosis.

Immunostaining for collagen IV in the control group showed single, thin, continuous mesothelial basement membrane. Light microscopy showed numerous, well organized collagen fibers in peritoneal *lamina propria*, particulary noticeable when stained for collagen IV (Figure 1).

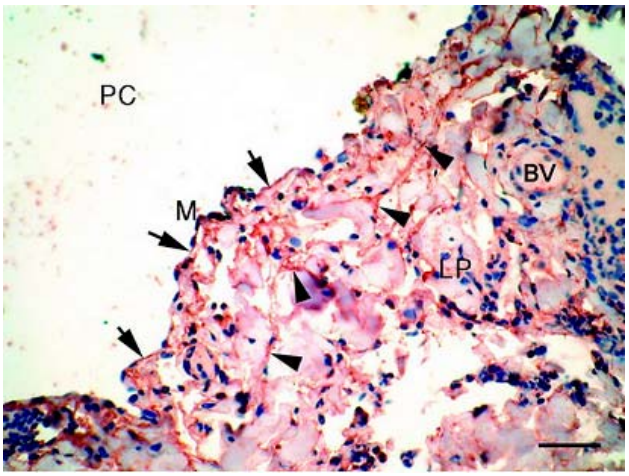


Fig. 1 – Healthy human parietal peritoneum (light micrograph)

LP – *lamina propria*; M – mesothelium; PC – peritoneal cavity; BV – blood vessel. Mesothelial basement membrane (arrows) and *lamina propria* (arrowheads) immunostained for collagen IV (scale bar = 30 μm)

Blood vessel walls in the end–stage renal disease patients showed positive immunostaining for collagen IV (Figure 2) but, with magnification 300 ×, neither qualitative nor quantitative alterations in collagen IV distribution were observed, compared to the healthy subjects.

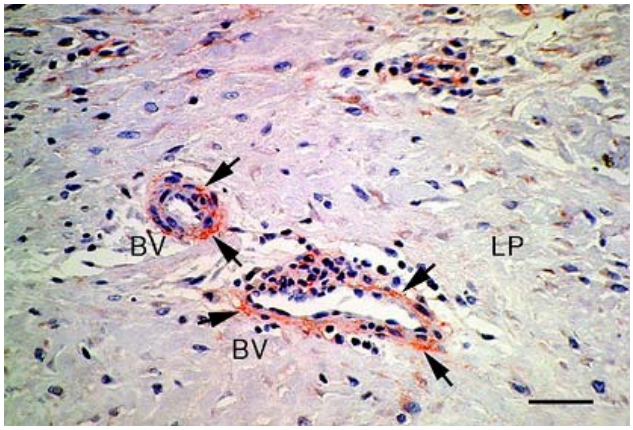


Fig. 2 – Parietal peritoneum in end-stage renal disease patients (light micrograph)

LP – *lamina propria*; Arrows show immunopositive reaction for collagen IV in blood vessels (BV) walls (scale bar = 30 μm)

In the patients on peritoneal dialysis even with light microscopy we observed numerous sites of mesothelial detachment from peritoneal lamina propria, especially well noticeable when stained for collagen IV in mesothelial basement membrane (Figure 3a), which is frequently duplicated in the PD patients (Figure 3b). Blood vessel walls showed positive immunostaining for collagen IV (Figure 3c).

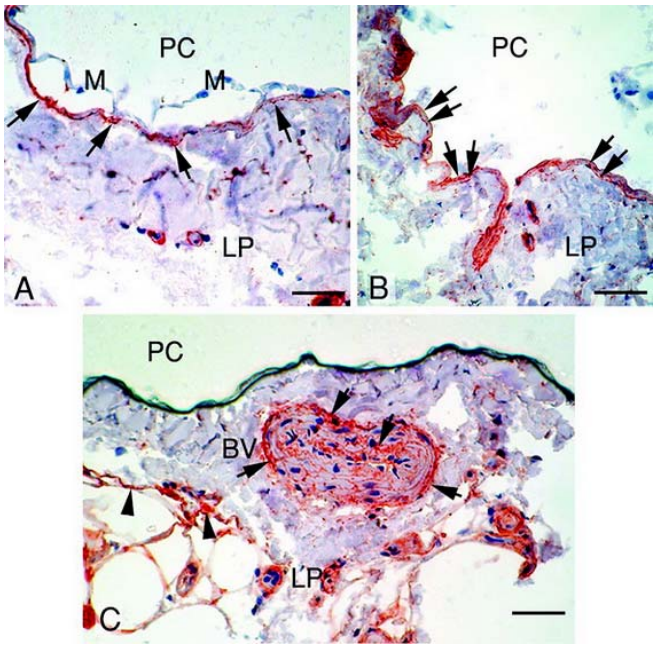


Fig. 3 – Parietal peritoneum in patients on peritoneal dialysis immunostained for collagen IV

(scale bar = 30 μm): LP – *lamina propria*; M – mesothelium; PC – peritoneal cavity. A) Mesothelial detachment from basement membrane (arrows); B) Duplication of mesothelial basement membrane (arrows); C) Collagen IV in blood vessels (BV) wall (arrows) and connective tissue (arrowheads).

On morphometric analysis with magnification 300 ×, it was clearly visible that peritoneal blood vessels in the PD patients had significantly thicker walls than blood vessels of the healthy subjects (48.43 μm *vs* 28.98 μm; $p < 0.01$). Furthermore, blood vessel walls were significantly thicker in the PD patients than in the ESRD patients (48.43 μm *vs* 35.90 μm; $p < 0.05$), as shown in Figure 4.

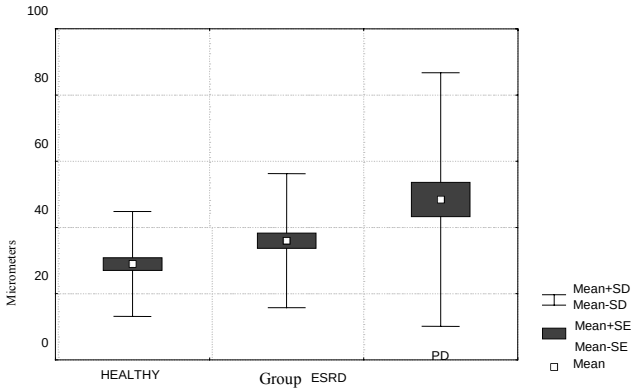


Fig. 4 – Peritoneal blood vessels wall thickness

ESRD – end-stage renal disease; PD – peritoneal dialysis

When analysing distribution of blood vessels with regard to wall thickness, we observed the vessels with extremely thick walls in the PD patients group, shifting the curve to the right. Both the healthy subjects and the ESRD patients had normal distribution of blood vessels with regard to wall thickness (Figure 5).

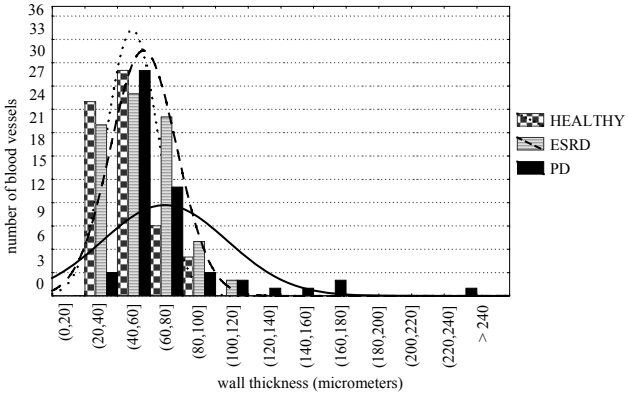


Fig. 5 – Peritoneal blood vessels distribution regarding wall thickness

ESRD – end-stage renal disease; PD – peritoneal dialysis

Discussion

In the present study of the human peritoneum, special attention was payed to immunohistochemical characteristics of elements of submesothelial connective tissue. We demonstrated pathological alterations in peritoneal submesothelial interstitial tissue and in mesothelial and endothelial basement membranes, using positive immunoreaction for collagen IV in these tissue elements in the renal disease patients.

Previous immunohistochemical studies of the peritoneum showed fascicular pattern of collagen IV, fibronectin and laminin fibers and diffuse fibers of collagen I in the submesothelial interstitial tissue [14]. The peritoneal mesothelium and endothelium of peritoneal blood vessels in healthy persons have single basement membranes. They represent a barrier for blood proteins and support for mesothelial and endothelial cells. Positive immunoreaction for collagen IV was found in mesothelial and endothelial basement membrane.

Uremic alterations of serous membranes in the ESRD patients affect the peritoneum as, well. Peritoneal tissue is highly delicate and extremely sensitive. Changes of internal environment, inflammation or minor injuries cause cell degeneration and desquamation. Uremic serositis itself causes detachment of mesothelial cells from their basement membrane and cell death. Mesothelial regeneration is *conditio sine qua non* for maintaining normal physiological functioning of the peritoneum in ESRD, and especially when PD is performed. Denuded regions are colonized with new mesothelial cells of yet undetermined origin. Several possible sources have been proposed: precursors from the bone marrow, mature undamaged mesothelial cells from adjacent and/or opposite peritoneal areas, precursors from *lamina propria* or free mesothelial cells from peritoneal fluid [15].

Detailed follow-up of mesothelial regeneration process, cells' origin and determination of their maturity is possible with immunohistochemical staining of mesothelial cytoskeleton (actin microphylaments, microtubules and intermediary desmin, vimentin and cytokeratin filaments) and determining the amount of cytokeratine and vimentin. Especially important is the presence of cytokeratin 8 and 18, which are specific markers for mesothelial cells [3]. Elements of the mesothelial cytoskeleton may also serve as markers for cell maturity. Young mesothelial cells in culture have equal amount of low molecular mass cytokeratin and vimentin, but during maturation the amount of vimentin rises. Mature mesothelial cells have high molecular mass cytokeratin and vimentin fibers [15].

The peritoneum suffers various structural alterations during PD. Destruction of mesothelium presents as: decrease in number or complete loss of microvilli, decrease in number of pinocytotic vesicles, widening and degeneration of rough endoplasmic reticulum, mitochondrial picnosis, apical cytoplasmatic protrusions and paracrystalline inclusions in these cells are obseved with electron microscopy [3, 16–19].

In physiological conditions, basal surfaces of mesothelial cells are in contact with basal lamina which they produce. Electron microphotographs clearly demonstrate *lamina densa* and light layer of *lamina lucida* above it, separating it from mesothelial cells. Immediately beneath *lamina densa* are collagen fibers organized in fascicular pattern[3]. Collagen is synthesized and secreted by fibroblasts from peritoneal connective tissue to form *lamina fibroreticularis*. Collagen fibers can be clearly detected with light microscopy with specific immunostaining. In the present study, immunostaining for collagen IV in the PD patients allowed visualisation of detachment of mesothelium from its basement membrane (and consecutive denudement of submesothelial layer) even with basic light microscopy.

A valuable finding in the PD patients is duplication (multiplication) of the mesothelial basement membrane. This was previously observed with electron microscopy [3, 15]. We already found this diabetiformic alteration of the basement membrane using electron microscopy, but in this study we showed that it can also be seen with light microscopy when immunohistochemical staining for collagen IV in the mesothelial basement membrane is applied [6]. This may enable a routine follow-up of peritoneal membrane status during PD.

Conclusion

Basic microscopy techniques, such as light microscopy, and relatively low-budget preparation methods (immunohistochemistry) may contribute significantly in studying peritoneal membrane in the ESRD and PD patients. Data obtained using standard antibodies for certain peritoneal structures (collagen IV in mesothelial and endothelial basement membranes) which suffer alterations in ESRD or during PD, are valuable in assessing adequacy of the peritoneal membrane in filtration and secretion processess it performs. Various degrees of damage to the peritoneal membrane represent clinical indication for PD cessation and transfer to hemodialysis.

On the other hand, timely detection of moderate peritoneal tissue alterations allows undertaking adequate measures (such as changing dialysate or temporary cessation of PD) in order to prevent a complete deterioration of peritoneal membrane, irreversible damage and unusability for PD.

Aknowledgements

This study was supported by the Ministry of Science and Environmental Protection of the Republic Serbia, Grant No 145070B.

R E F E R E N C E S

1. *Gotloib L, Shoshtak A.* Functional structure of the peritoneum as a dialysing membrane. In: *Gokal R, Khanna R, Krediet RTh, Nolph KD*, editors. Textbook of peritoneal dialysis. 2nd ed. Dodrecht, Boston, London: Kluwer Academic Publishers; 2000. p. 667–708.
2. *Trpinac D, Stojimirović B, Obradović M, Milutinović D, Obradović D, Nešić V.* Effect of uremia and peritoneal dialysis on peritoneal mesothelial cells Vojnosanit Pregl 2002; 59(1): 17–21. (Serbian)
3. *Di Paolo N, Sacchi G.* Atlas of peritoneal histology. Perit Dial Int 2000; 20(suppl 3): 6–87.
4. *Obradović M, Stojimirović B, Trpinac D, Milutinović D, Obradović D, Nešić V.* Ultrastructural changes of peritoneal lining cells in uremia. Adv Perit Dial 2000; 16: 26–30.
5. *Stojimirović B, Obradović M, Trpinac D, Milutinović D, Obradović D, Nešić V.* Mesothelial paracrystalline inclusions in continuous ambulatory peritoneal dialysis patients. Perit Dial Int 2001; 21 Suppl 3: 54–7.
6. *Stojimirović B, Trpinac D, Obradović M, Milutinović D, Obradović D, Nešić V.* Changes in peritoneal mesothelial cells in patients on peritoneal dialysis. Med Pregl 2001; 54(5–6): 219–23. (Serbian)
7. *Gotloib L, Digenis GE, Rabinovich S, Medline A, Oreopoulos DG.* Ultrastructure of normal rabbit mesentery. Nephron 1983; 34(4): 248–55.
8. *Hoff CM.* Ex vivo and in vivo gene transfer to the peritoneal membrane in a rat model. Nephrol Dial Transplant 2001; 16(3): 666–8.
9. *Buemi M, Aloisi C, Cutroneo G, Nostro L, Favaloro A.* Flowing time on the peritoneal membrane. Nephrol Dial Transplant 2004; 19(1): 26–9.
10. *Di Paolo N, Sacchi G, De Mia M, Gaggiotti E, Capotondo L, Rossi P,* et al. Morphology of the peritoneal membrane during continuous ambulatory peritoneal dialysis. Nephron 1986; 44(3): 204–11.
11. *Dobbie JW.* Peritoneal ultrastructure and changes with continuous ambulatory peritoneal dialysis. Perit Dial Int 1993; 13 Suppl 2: S585–7.
12. *Miettinen M, Kovatich AJ.* HBME-1: a monoclonal antibody useful in the differential diagnosis of mesothelioma, adenocarcinoma, and soft tissue and bone tumors. Appl Immunohistochem 1995; 3(2): 115–22.
13. *Boenisch T.* Handbook Immunochemical Staining Methods. 3rd ed. CA, USA Carpinteria: DakoCytomation; 2001.
14. *Witz CA, Montoya-Rodriguez IA, Cho S, Centonze VE, Bonewald LF, Schenken RS.* Composition of the extracellular matrix of the peritoneum. J Soc Gynecol Investig 2001; 8(5): 299–304.
15. *Dobbie JW.* Ultrastructure and the pathology of the peritoneum in peritoneal dialysis. In: *Gokal R, Nolph KD,* editors. The textbook of peritoneal dialysis. Dordrecht: Kluwer Academic Publishers; 1994. p. 17–44.
16. *Bertoli SV, Barone MT, Vago L, Bonetto S, De Vecchi A, Scalamogna A*, et al. Changes in peritoneal membrane after continuous ambulatory peritoneal dialysis-a histopathological study. Adv Perit Dial 1999; 15: 28–31.
17. *Mateijsen MA, van der Wal AC, Hendriks PM, Zweers MM, Mulder J, Struijk DG,* et al. Vascular and interstitial changes in the peritoneum of CAPD patients with peritoneal sclerosis. Perit Dial Int 1999; 19(6): 517–25.
18. *Coles GA, Topley N.* Long-term peritoneal membrane changes. Adv Ren Replace Ther 2000; 7(4): 289–301.
19. *Fang W, Qian JQ, Yu ZY, Chen SS.* Morphological changes of the peritoneum in peritoneal dialysis patients. Chin Med J (Engl) 2004; 117(6): 862–6.

Received on December 29, 2009.
Accepted on May 4, 2010.

ORIGINALNI ČLANAK

UDC: 616.36-091.8-092.9:615.099]:612.014.43
DOI: 10.2298/VSP1107561M

Uticaj temperature okoline na hepatocelularno oštećenje kod pacova nakon unošenja 3,4-metilendioksimetamfetamina

Ambient temperature impact on hepatocellular liver damage in rats following intake of 3,4-methylenedioxymethamphetamine

Nadica S. Marinković*, Živorad Maličević†, Jovan Dimitrijević*

Vojnomedicinska akademija, *Institut za patologiju i sudsku medicinu, †Institut za medicinska istraživanja, **Beograd, Srbija**

Apstrakt

Uvod/Cilj. Poznato je da je 3,4-metilendioksimetamfetamin (MDMA, *Ecstasy*) psihostimulativno sredstvo. Najčešće se unosi oralno, u vidu tableta. Resorbuje se preko sluzokože digestivnog trakta. Najčešći klinički znak uzimanja MDMA je hipertermija. Smrtna trovanja nakon uzimanja MDMA na obdukciji najčešće pokazuju infarkt miokarda ili cerebrovaskularno krvarenje. Međutim, opisana su i oštećenja jetre i bubrega. Cilj ove studije bio je da se utvrdi da li temperatura okoline utiče na oštećenje jetre pacova nakon primene MDMA. **Metode.** Eksperiment je trajao 8 i 24 h na temperaturama 12, 22 i 32°C. Praćeni su biohemijski parametri (bilirubin, ALT, AST, AP, gama GT i LDH) i patohistološke promene jetre. **Rezultati.** Rezultati su pokazali da su oštećenja jetre najveća na temperaturi od 32°C. Oštećenja jetre su se manifestovala kao portalna inflamacija, periportalna nekroza, lobularna nekroza, staza, intralobularno krvarenje i porast enzima jetre u krvi. **Zaključak.** Oštećenje jetre nakon primene MDMA raste sa porastom temperature okoline, a najizraženije je na temperaturi od 32°C.

Ključne reči:
n-metil-3,4-metilendioksiamfetamin; jetra; pacovi; histologija; temperatura.

Abstract

Bacground/Aim. 3,4-methylendioxymethamphetamine (MDMA, Ecstasy) is a psycho-stimulating agent. It is usualy taken orally in the form of tablets. It is absorbed throught the gastrointestinal mucous membrane. Hyperthermia is the most prominent clinical sign of MDMA intake. The most prominent forensic finding of lethal MDMA poisoning is myocardial infarction and cerebrovascular bleeding. However, liver and kidney damage: have also been described. The aim of this research was to determine if ambient temperatures affect liver damage in the experimental rats. **Methods.** The experiment was conducted for 8 h and 24 h, at temperatures of 12°C, 22°C and 32°C. Both biochemical parameters (ALT, AST, AP, gamma GT and LDH) and pathohistological changes of the liver were monitored. **Results.** Our reserch demonstrated that the most serious lever damage occurred at 32°C. Liver damage was manifested as portal inflammation, periportal necrosis, lobular necrosis, stasis, intralobular hemorrhage and incerease of liver enzymes serum activity. **Conclusion.** Liver damage after MDMA intake rises with the increase of ambient temperature, and it is most pronounced at the temperature of 32°C.

Key words:
3,4-methylenedioxyamphetamine; liver; rats; histology; temperature.

Uvod

Derivat metamfetamina, 3,4-metilendioksimetamfetamin (MDMA) razlikuje se od matičnog jedinjenja prisustvom metil grupe. Pripada grupi tzv. „dizajniranih droga“, a poznatiji je kao *Ecstasy*. Unosi se najčešće peroralno, u obliku tableta, ređe kapsula, retko ušmrkavanjem praha, a najređe intravenski. Resorbuje se preko sluzokože digestivnog trakta. Liposolubilan je i lako prolazi hematoencefalnu barijeru. Maksimalni nivo u plazmi postiže 1,8–2,4 h nakon oralnog unošenja. Poluživot eliminacije ovog jedinjenja je 7,7–8,6 h [1].

Vezivanjem metil grupe za amino grupu pojačavaju se stimulativna, a smanjuju halucinogena svojstva amfetamina; 3,4-metilendioksimetamfetamin izaziva euforiju, dobro raspoloženje, povećanje osetljivosti svih čula, smanjenje anksioznosti. Takođe, pojačava osećaj fizičke snage, čime omogućava „maratonsko“ igranje korisnika u diskotekama, kao i libido, te se naziva „pilula ljubavi“. Olakšava kontakte među ljudima. Otklanja umor i pospanost, a nesanica izazvana njime može trajati danima [1, 2].

Najčešći neželjeni efekti primene MDMA su hipertermija, hipertenzija, hipotenzija, fokalni neurološki ispadi, ta-

Correspondence to: Nadica Marinković, Vojnomedicinska akademija, Institut za patologiju i sudsku medicinu, Crnotravska 17, 11 000 Beograd, Srbija. Tel.: +381 11 3608 342. E-mail: nadicamarinkovic@yahoo.com

hipneja, srčana aritmija, vazokonstrikcija. Obdukcioni nalazi su nespecifični. Opisana su različita ošetećenja gotovo svih organa, a najčešće jetre i nervnog sistema [2–4]. Oštećenja nervnog sistema su na nivou pražnjenja depoa 5-hidroksitriptamina (5-HT) i potvrđeno je da su izražena tek na temperaturama okoline iznad 26°C [5, 6]. Oštećenje jetre ide od masne promene, preko hepatitisa do fulminantnog oštećenja koje zahteva transplantaciju [7]. Od laboratorijskih analiza opisan je visok nivo serumskih transaminaza. Dolazi do povećanja permeabilnosti membrana, smanjenja kapaciteta za sintezu i uskladištenje materija, izmene ekskretorne i detoksikacione funkcije jetre, povećanja mezenhimalne reakcije i abnormalne imunološke reakcije [8, 9]. Nivo aktivnosti enzima u plazmi zavisi od mogućnosti jetre da sintetiše proteine, s obzirom na to da se specifični plazmatski enzimi sintetišu i izlučuju iz jetre. U slučaju akutnog oštećenja, usled metaboličke insuficijencije parenhimskih ćelija, dolazi do izmene i ove funkcije jetre. Izgled enzimskog profila i njegov dijagnostički značaj zavise i od poluvremena života enzima u cirkulaciji. Enzimi sa dužim poluvremenom života dostižu maksimalnu aktivnost u serumu kasnije i akumuliraju se sa snižavanjem nivoa enzima u toku akutnog oštećenja. Od enzima koji su značajni za procenu funkcije jetre relativno kratko poluvreme života imaju aspartat aminotransferaza (AST) i laktat dehidrogenaza (LDH), a oko tri puta duže ima alanin aminotransferaza (ALT). Aktivnosti ALT i AST praktično su jednake u citoplazmi ćelija jetre, dok se oko 40% AST nalazi u mitohondrijama. U slučaju kada je AST viša od ALT, kao kod trovanja, znači da su i membrane mitohondrija oštećene. Promene na mitohondrijama javljaju se tokom 30 minuta, a gotovo potpuno se razvijaju tokom jednog sata. Pošto se AST u serumu inaktivira mnogo brže od ALT, veći porast AST u odnosu na ALT govori da je bolest ili oštećenje verovatno još uvek u akutnoj fazi. Povišene vrednosti AST u serumu, kao mitohondrijalnog enzima, ukazuje na akutnu nekrozu ćelija jetre. Povišene vrednosti gama glutamil transferaze (GT) ukazuju na holestazu ili toksična oštećenja jetre koja stimulišu sintezu enzima vezanih za membranu [10].

Cilj ovog istraživanja bio je da se ustanovi da li različite temperature okoline utiču na stepen oštećenja jetre pacova nakon uzimanja MDMA.

Metode

Kao eksperimentalne životinje odabrani su pacovi soja Wistar, muškog pola, starosti oko šest nedelja, prosečne težine 258 g, u skladu sa etičkim principima rada sa laboratorijskim životinjama koji striktno poštuju pravila data u *European Community Guidelines* (EEC Directive) iz 1986.

Sadržaj tablete analiziran je kvalitativno infracrvenom spektrofotometrijom, tankoslojnom i gasnom hromatografijom upotrebom „FTIR-8300“ i „GC-17A“ marke „Shimadzu“, Japan, i kvantitativno-gasnom hromatografijom sa FID detektorom. Ustanovljeno je da tablete sadrže 15,68% 3,4-metilendioksimetamfetamina. Ostali deo tablete sadržao je neaktivnu supstancu, uglavnom laktozu. Tablete su bile rastvarane u destilovanoj vodi.

Životinje su bile podeljene u grupe od po osam jedinki u svakoj. Bilo je šest kontrolnih grupa, šest grupa koje su dobijajale po 20 mg/kg MDMA i šest grupa koje su dobijale po 40 mg/kg MDMA, od kojih je po jedna grupa bila podvrgnuta 8-časovnom ili 24-časovnom boravku u komorama na temperaturi od 12, 22 ili 32°C. Svaka životinja kontrolne grupe dobila je po 1 mL destilovane vode, a životinje u eksperimentalnim grupama po 1 mL rastvora MDMA u destilovanoj vodi u dozi od 20 mg/kg, odnosno 40 mg/kg. Svim životinjama rastvor je dat gastričnom sondom, peroralno. Nakon isteka vremena predviđenog za eksperiment, životinje su anestezirane etrom i uzeta im je krv iz srca radi određivanja biohemijskih parametara. Praćeni su sledeći biohemijski parametri u serumu: bilirubin, AST, ALT, alkalna fosfataza (AP), LDH i gama GT. Biohemijska analiza vršena je na aparatu Siemens-ex Dade Beuring, tipa R×L MAX, Nemačka. Nakon uzimanja krvi, anestezirane životinje su žrtvovane i uzet im je desni režanj jetre radi praćenja patohistoloških (PH) promena. Isečci organa fiksirani su u 5% neutralnom puferisanom formalinu, kalupljeni u parafinu, serijski sečeni rotacionim mikrotomom Leica RN2135, Nemačka, debljine 5 μm i bojeni metodom hematoksilin-eozin i Masson trihrom. Obojeni PH preparati posmatrani su svetlosnim mikroskopom Leica DMLB, Nemačka.

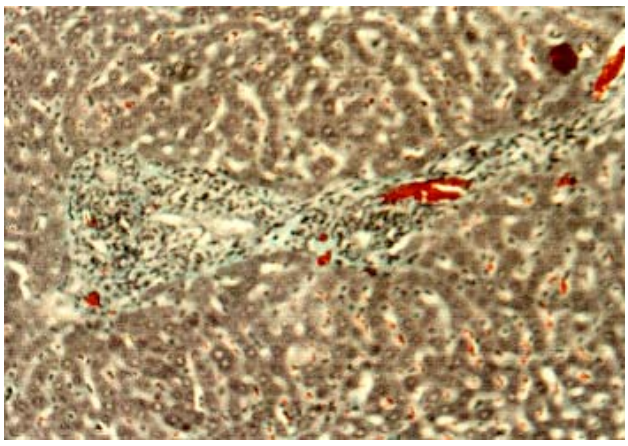
Patohistološkom analizom obuhvaćeni su sledeći parametri: portalna inflamacija, periportalna nekroza, fibroza, oštećenje žučnih kanalića, prominencija Kupffer-ovih i endotelnih ćelija, mononuklearne ćelije u sinusoidima, acidofilna (apoptotična) telašca, vaskulitis (vaskulopatija), intravaskularna tromboza (koagulopatija), staza, intralobularna hemoragija, anizocitoza, anizonukleoza, vakuolizacija nukleusa, pigmenti (lipofuscin) i dilatacija sinusoida.

Vrednosti praćenih biohemijskih parametara obrađene su metodama deskriptivne statistike i predstavljene kao srednja vrednost i standardna devijacija. Za statističku analizu korišćen je Studentov *t*-test, mediana test i Kruskall-Wallisov test. Sve razlike na nivou $p < 0,05$ uzete su kao statistički značajne.

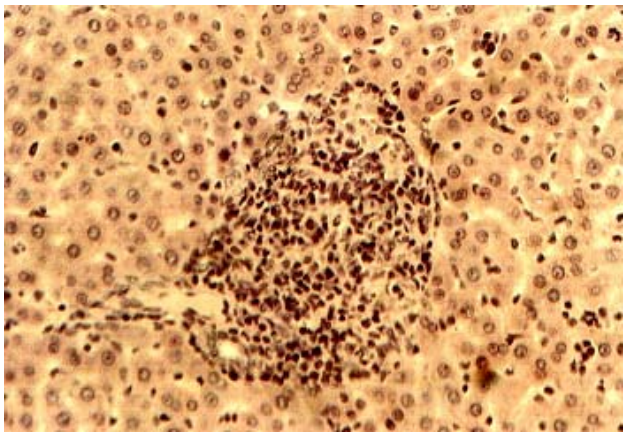
Rezultati

Kod životinja kontrolne grupe koje su boravile 8 h na sobnoj temperaturi od 22°C nije bilo portalne inflamacije, dok kod većine životinja u obe eksperimentalne grupe (20 i 40 mg/kg MDMA) došlo je do lake portalne inflamacije. Statistički značajna razlika nađena je u 8-časovnom eksperimentu kod životinja koje su primile dozu od 20 mg/kg MDMA u odnosu na kontrolnu grupu ($p < 0,0012$), i grupu koja je primila dozu od 40 mg/kg MDMA ($p < 0,001$). U 24-časovnom eksperimentu, takođe, postojala je statistički značajna razlika između kontrolne grupe i grupa koje su dobile MDMA u dozi od 20 mg/kg i 40 mg/kg ($p < 0,03$, odnosno $p < 0,05$).

Na temperaturi okoline od 12°C u 8-časovnom eksperimentu postojala je statistički značajna razlika u stepenu portalne inflamacije za dozu od 40 mg/kg MDMA ($p < 0,0009$), dok u 24-časovnom eksperimentu nije bilo statističke značajnosti. Na sobnoj temperaturi od 22°C nije bilo statistički značajne razlike u stepenu izraženosti portalne inflamacije između kontrolne i eksperimentalnih grupa (slike 1 i 2).



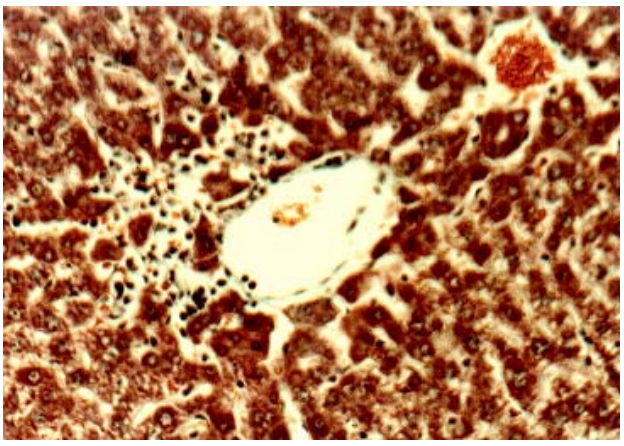
Sl. 1 – Prošireni portni prostor sa mononuklearnim ćelijskim infiltratom i vezivnim vlaknima koji ne prelaze njegove granice (eksperimentalna grupa 20 mg MDMA, 22°C, 8 h; Masson trihrom, ×150)



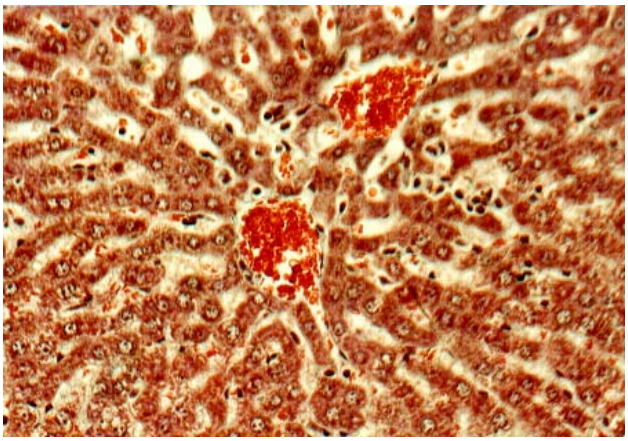
Sl. 2 – Prošireni portni prostor sa izraženim polimorfnim celularnim infiltratom koji probija laminu limitans i zahvata okolne hepatocite (eksperimentalna grupa 40 mg MDMA, 22°C, 24 h; HE, ×250)

U 8-časovnom eksperimentu na temperaturi od 22°C, pojavila se laka periportalna nekroza. Ona je ustanovljena kod dve životinje iz kontrolne grupe, dve iz eksperimentalne grupe koja je dobila 20 mg/kg MDMA, i kod četiri životinje iz grupe koja je dobila 40 mg/kg MDMA. U 24-časovnom eksperimentu na sobnoj temperaturi od 22°C nije uočena periportalna nekroza. Na temperaturi od 12°C nije ustanovljena periportalna nekroza. Na temperaturi okoline od 32°C u 8-časovnom eksperimentu centrolobularna nekroza uočena je kod četiri životinje koje su dobile 20 mg/kg MDMA, a u 24-časovnom eksperimentu kod jedne životinje koja je dobila 40 mg/kg MDMA (slike 3 i 4).

Lobularna nekroza javila se na temperaturi od 32°C tokom 8-časovnog eksperimenta kod eksperimentalne grupe koja je dobila 40 mg/kg MDMA ($p < 0,02$). Tokom 24-časovnog eksperimentu na temperaturi okoline od 32°C stepen lobularne nekroze bio je izraženiji kod kontrolne grupe. Prominencija Kupffer-ovih ćelija bila je izražena na svim ispitivanim temperaturama i kod kontrolnih i kod eksperimentalnih grupa.



Sl. 3 – Centrolobularna nekroza hepatocita (eksperimentalna grupa 20 mg MDMA, 32°C, 8 h; Masson trihrom, ×200)



Sl. 4 – Centrolobularni ćelijski infiltrat (eksperimentalna grupa 40 mg MDMA, 32°C, 24 h; Masson trihrom, ×200)

Većina životinja u obe eksperimentalne grupe pokazivala je laku ili umerenu stazu. Statistički značajna razlika u stepenu staze postojala je na sobnoj temperaturi od 22°C i u 8-časovnom ($p < 0,0001$) i 24-časovnom eksperimentu ($p < 0,0007$) u grupi koja je dobila dozu od 40 mg/kg MDMA. Na ostalim temperaturama (12 i 32°C) razlika nije bila statistički značajna.

Kod životinja koje su dobile MDMA ustanovljeno je i intralobularno krvarenje. Na temperaturi od 32°C krvarenje se javilo i kod grupe koja je dobila 20 mg/kg MDMA i kod grupe koja je dobila 40 mg/kg MDMA. Statistički značajna razlika u pojavi krvarenja tokom 8-časovnog eksperimenta javila se u grupi koja je dobila 20 mg/kg MDMA ($p < 0,02$), a u 24-časovnom eksperimentu u grupi koja je dobila 40 mg/kg MDMA ($p < 0,05$).

Na temperaturi okoline od 22°C krvarenje je nastalo samo u 24-časovnom eksperimentu u grupi koja je dobila 40 mg/kg MDMA, sa statističkom značajnošću $p < 0,005$. Na temperaturi od 12°C hemoragija se javila samo kod jedne eksperimentalne životinje koja je dobila 20 mg/kg MDMA.

Anizocitoza se ispoljavala u kontrolnim i nešto izraženije u eksperimentlanim grupama. Nađena je, ipak, statistički značajna razlika u stepenu izraženosti kod eksperimentalnih grupa u od-

nosu na kontrolnu. Ta razlika bila je statistički značajna na sobnoj temperaturi od 22°C u 8-časovnom ($p < 0,001$), kao i u 24-časovnom eksperimentu ($p < 0,0001$) uz primenu doze od 40 mg/kg MDMA. Na temperaturi okoline od 12°C nađena je statististički značajna razlika u 8-časovnom eksperimentu kod doze od 40 mg/kg MDMA ($p < 0,004$), dok u 24-časovnom eksperimentu razlika nije bila statistički značajna. U eksperimentu na 32°C nađena je statistički značajna razlika u izraženosti anizocitoze kod doze od 40 mg/kg MDMA i u 8-časovnom ($p < 0,01$) i u 24-časovnom eksperimentu ($p < 0,04$).

Anizonukleoza je, takođe, ustanovljena kod kontrolne, i nešto izraženija kod eksperimentalnih grupa. Ta razlika pokazala je statističku značajnost na sobnoj temperaturi od 22°C u 8-časovnom eksperimentu ($p < 0,01$), kao i u 24-časovnom eksperimentu ($p < 0,0001$). Statistički, bila je značajna i razlika u izraženosti anizonukleoze između kontrolne i grupa koje je dobila 40 mg/kg MDMA tokom 8-časovnog eksperimenta na temperaturi okoline od 32°C ($p < 0,005$).

Aktivnost AST pokazala je na svim ispitivanim temperaturama (12°C, 22°C, 32°C) statistički značajan porast vrednosti u eksperimentalnim grupama i u 8-časovnom i 24-časovnom eksperimentu (tabele 1 i 2). Najveći porast aktivnosti AST uočen je na temperaturi od 32°C tokom 8-časovnog eksperimenta u grupi koja je dobila 40 mg/kg MDMA.

Aktivnost ALT, na temperaturi od 22°C, pokazala je pad u eksperimentalnoj grupi koja je dobila 40 mg MDMA tokom 8-časovnog eksperimenta ($p < 0,001$).

Na temperaturi od 32°C i tokom 8-časovnog, kao i 24-časovnog eksperimenta uočen je značajan porast vrednosti ALT u odnosu na kontrolnu grupu ($p < 0,03$ za grupu koja je dobila dozu od 20 mg/kg MDMA i $p < 0,004$ za grupu koja je dobila dozu od 40 mg/kg MDMA).

Aktivnost ALT bila je najveća kod eksperimentalnih grupa koje su dobile 40 mg/kg MDMA i koje su bile izložene temperaturi od 32°C tokom 8 h i 24 h (tabele 1 i 2).

Serumska aktivnost LDH kod životinja koje su dobile 40 mg/kg MDMA i bile izložene temperaturi od 22°C tokom 8 h i 24 h bila je statistički značajano viša u odnosu na vrednosti kontrolne grupe (tabele 1 i 2). Aktivnost LDH bila je viša u odnosu na kontrolne vrednosti kod životinja tretiranih sa obe doze MDMA. U izloženih temperaturi od 32°C tokom 8 h i 24 h (tabela 1).

Gama GT, na sobnoj temperaturi od 22°C, imala je porast serumske aktivnosti u grupi koja je dobila 40 mg MDMA u 8-časovnom eksperimentu (tabela 1). Na istoj temperaturi, u toku 24 h, vrednosti gama GT su bile povećane kod eksperimentalne grupe koja je dobila 20 mg/kg MDMA (tabela 2). Statistički, bio je značajan porast vrednosti gama GT u 8-časovnom eksperimentu na temperaturi 32°C kod grupe koja je dobila 40 mg/kg MDMA ($p < 0,035$) (tabela 1).

MDMA niti na jednoj od ispitivanih ambijentalnih temperatura, ni u 8-časovnom, niti u 24-časovnom eksperimentu nije uticao na vrednost AP u serumu eksperimentalnih životinja (tabele 1 i 2).

Tabela 1

Aktivnost enzima kontrolne (KG) i eksperimentalnih grupa (EG) u 8-časovnom eksperimentu

Grupe i doza primljenog MDMA, temperatura okoline	AST (U/L) min-max ± SD	ALT (U/L) min-max ± SD	LDH (U/L) min-max ± SD	γ-GT (U/L) min-max ± SD	AP (U/L) min-max ± SD
KG, 22°C	106–293 ±148,71	64–103 ±12,88	74–1047 ±180,96	5–9 ± 1,29	262–332 ± 31,40
EG, 20 mg/kg, MDMA, 22°C	134–298 ± 68,27*	51–94 ± 15,68	126–962 ± 287,47**	6–10 ± 1,60	172–378 ± 63,10
EG, 40 mg/kg, MDMA, 22°C	157–682 ± 213,67**	42–115±25,76**	448–1304±272,24**	6–9 ± 0,99**	199–336 ± 42,15
KG 12°C	114–159 ± 23,77	55–86 ± 10,89	172–956 ± 281,34	5–9 ± 1,41	234–373 ± 59,60
EG, 20 mg/kg, MDMA, 12°C	152–302 ± 47,6**	46–78 ± 11,41	347–1012 ± 230,91	6–10 ± 1,60	215–386 ± 63,85
EG, 40 mg/kg, MDMA, 12°C	131–565 ± 142,57**	51–92 ± 15,09	152–316 ± 64,54	6–9 ± 1,06	148–324 ± 54,14
KG 32°C	118–182 ± 22,32	55–82 ± 8,94	327–1094 ± 282,93	5–9 ± 1,19	213–366 ± 52,44
EG, 20 mg/kg, MDMA, 32°C	10–1194 ± 426,70*	60–166 ± 46,23*	568–2268 ± 646,76**	6-40 ± 13,23	9–700 ± 202,82
EG, 40 mg/kg, MDMA, 32°C	386–1671 ±496,31**	75–337±97,54**	391–2229 ± 638,97**	6-23 ± 5,27*	223–677 ± 133,00

***p < 0,05 u odnosu na kontrolnu grupu na istoj temperaturi okoline**
****p < 0,005 u odnosu na kontrolnu grupu na istoj temperaturi okoline**
MDMA – 3,4-metilendioksimetamfetamin; AST – aspartat aminotransferaza; ALT – alanin aminotransferaza; LDH – laktat dehidrogenaza; γ-GT – gama glutamil transferaza; AP – alkalna fosfataza

Tabela 2

Aktivnost enzima kontrolne (KG) i eksperimentalnih grupa (EG) u 24-časovnom eksperimentu

Grupe i doza primljenog MDMA sobna temperatura	AST (U/L) min-max ± SD	ALT (U/L) min-max ± SD	LDH (U/L) min-max ± SD	γ-GT (U/L) min-max ± SD	AP (U/L) min-max ± SD
KG, 22°C	118–211 ± 31,44	63–90 ± 9,38	410–905 ± 198,38	5–8 ± 1,19	268–443 ± 59,86
EG, 20 mg/kg, MDMA, 22°C	129–801 ± 227,98**	61–184 ± 42,45	282–1086 ± 238,29	6–9 ± 1,16*	215–443 ± 73,85
EG, 40 mg/kg, MDMA, 22°C	261–876 ± 32,93**	68–154 ± 9,54	941–2379±451,98**	6–9 ± 1,10	8–418 ± 44,41
KG, 12°C	114–263 ± 46,99	73–113 ± 13,77	174–925 ± 267,10	4–7 ± 1,07	205–358 ± 63,39
EG, 20 mg/kg, MDMA 12°C	121–984 ± 36,25**	53–149 ± 8,39	448–1216 ± 254,46	4–7 ± 1,13	164–339 ± 60,97
EG, 40 mg/kg, MDMA 12°C	141–616 ± 151,51**	69–106 ± 15,28	252–11164 ± 294,00	4–8 ± 1,28	186–351 ± 49,88
KG, 32°C	141–252 ± 34,79	59–82 ± 10,53	633–1646 ± 340,96	5–9 ± 1,39	237–357 ± 41,06
EG, 20 mg/kg, MDMA, 32°C	184–693 ± 232,03*	60–122 ± 34,44*	738–1691 ± 410,69	5–9 ± 1,41	251–387 ± 69,66
EG, 40 mg/kg, MDMA, 32°C	226–1376±494,22**	63–297±105,46**	684–1476±294,15**	7–9 ± 0,89	281–352±28,89

***p < 0,05 u odnosu na kontrolnu grupu na istoj temperaturi okoline**
****p < 0,005 u odnosu na kontrolnu grupu na istoj temperaturi okoline**
MDMA – 3,4-metilendioksimetamfetamin; AST – aspartat aminotransferaza; ALT – alanin aminotransferaza; LDH – laktat dehidrogenaza; γ-GT – gama glutamil transferaza; AP – alkalna fosfataza

Diskusija

U našem eksperimentu MDMA doveo je do toksične nekroze ćelija jetre, što je uslovilo porast aktivnosti AST i gama GT u serumu. Uočen je porast aktivnosti AST kod eksperimentalnih grupa u odnosu na kontrolne, na svim ispitivanim temperaturama, bez obzira na dozu MDMA.

Na temperaturi okoline od 32°C vrednosti AST su bile najviše, kao i patohistološke promene u vidu lobularne nekroze.

Aktivnost ALT pokazala je porast kod eksperimentalnih grupa u odnosu na kontrolne, bez obzira na dozu MDMA samo na temperaturi od 32°C.

Serumska aktivnost ALT, AST i LDH povećava se kada je ćelija do te mere oštećena da svi citoplazmatski enzimi izlaze u cirkulaciju. Povišena vrednosti AP javlja se kod opstrukcionih oboljenja jetre, hepatitisa, toksičnog delovanja lekova, Paget-ovog oboljenja, osteomalacije, rahitisa i malignih oboljenja jetre [10].

Upadljiv je porast vrednosti LDH u toku 24-časovnog eksperimenta uz primenu doze od 40 mg/kg MDMA i u toku 8-časovnog eksperimenta kod obe doze MDMA na temperaturi od 32°C. Isti enzim na sobnoj temperaturi od 22°C pokazuje izraziti porast vrednosti kod obe doze MDMA u 8-časovnim eksperimentu, dok u toku 24-časovnog eksperimenta ima porast samo kod primene veće doze MDMA. Evidentno je da i MDMA uslovljava porast enzimske aktivnosti, ali je i temperatura ta koja utiče na nivo ova dva enzima. Poznato je, naime, da su enzimske reakcije veoma osetljive na promenu temperature. Utvrđeno je da pri porastu temperature za 1°C dolazi do povećanja katalitičke aktivnosti enzima za oko 10%. Naime, kod toksičnog oštećenja jetre nema tipičnog enzimskog profila. Izmene serumskog enzimskog profila zavise od upotrebljenog agensa i jačine oštećenja. Veoma izrazito povišenje aktivnosti u serumu nalazi se kod oštećenja jetre sa ekstenzivnom nekrozom. U tim slučajevima aktivnosti enzima su 10 i 20 puta više u odnosu na aktivnost kod akutnog hepatitisa. Enzimski profil kod masivne nekroze jetre je najčešće LDH > AST > ALT, a kod hepatitisa ALT > AST > LDH [10]. Međutim, enzimski profil kod toksičnog oštećenja jetre može ukazivati na svaki tip oboljenja jetre.

Na osnovu različitih prikaza slučajeva sa oštećenjem niza organa usled primene MDMA, može se postaviti i pitanje da li je enzimski profil koji se pokazao u našem eksperimentu pokazatelj oštećenja jetre ili drugih organskih sistema u kojima se nalaze odgovarajući enzimi. Naime, aktivnost AST povećava se kod oštećenja srčanog mišića i jetre. Gama GT povećava se kod hepatobilijarnih oboljenja, ali se velike količine ovog enzima, pored jetre, nalaze i u bubrezima, pankreasu i prostati. Aktivnost LDH najveća je u bubrezima, zatim srcu, skeletnim mišićima, pankreasu, slezini, jetri, plućima i placenti. Enzim ALT nalazi se pretežno u jetri, a u manjim količinama u bubrezima, srcu, skeletnim mišićima i pankreasu. Alkalna fosfataza se nalazi u crevnoj sluznici, zatim placenti, bubregu, kostima, jetri, plućima i slezini. Međutim, eksperimentalna hipoksija izolovane jetre dovodi do povećanja aktivnosti enzima u krvi već nakon sedam minuta. U slučaju oštećenja koja su izazvana patološkim promenama na srcu, pankreasu ili prostati, aktivnost enzima u serumu povećava se tek nekoliko sati nakon oštećenja. U slučaju oštećenja skeletnih mišića enzimi se javljaju u serumu nakon nekoliko dana. Porast vrednosti AST, koji ima kratko vreme poluživota, u eksperimentu koji je trajao 8 ili 24 h, uz odgovarajuće morfološke promene jetre, ukazuje, ipak, da je njegovo poreklo iz jetre.

Patohistološkim pregledom isečaka jetre ustanovljeno je da su na temperaturi okoline od 32°C morfološke promene jetre najizraženije. Dominira lobularana nekroza, periportalna nekroza i lobularna hemoragija. Biohemijski parametri i patohistološke promene potvrđuju da visoka ambijentalna temperatura utiče na hepatotoksičnost MDMA. U toku 24 h, na temperaturi od 32°C pojavljuje se portalna inflamacija i lobularna nekroza i kod kontrolne grupe, sa manje izraženom stazom, bez hemoragije, što pokazuje da i sama visoka temperatura nakon određenog vremena dovodi do oštećenja jetre kod pacova.

Na temperaturi okoline od 12°C kod eksperimentalnih grupa, postoji samo izražena portalna inflamacija, periportalna nekroza nije ustanovljena, lobularna nekroza bila je prisutna samo kod jedne životinje, staza je bila manje ispoljena, a hemoragija postojala je samo kod dve životinje. To ukazuje da niska temperatura okoline ima zaštitni efekat kod oštećenja jetre nakon primene MDMA, a da visoka temperatura okoline pojačava hepatotoksičnost ovog jedinjenja.

Morfološke promene koje su ustanovljene kod životinja koje su dobile MDMA, bez obzira na dozu, su periportalna i lobularna nekroza koje su bile najizrazitije na temperaturi od 32°C. Hemoragija je najizraženija na temperaturi od 32°C, znatno manja na temperaturi od 22°C, ali izražene kod doze od 40 mg/kg MDMA. To ukazuje da se hepatotoksičnost koju ima *Ecstasy* povećava na višim temperaturama okoline, a da doza utiče samo na određene morfološke promene.

U dosadašnjim radovima, najčešće su opisivani hepatitis, masna promena i lobularna nekroza kao prikazi slučajeva, od oštećenja jetre izazvanih primenom MDMA [11–13]. Naše istraživanje potvrđuje postojanje inflamatorne reakcije na svim ispitivanim temperaturama, kao i nekrozu, koja je najizraženija na temperaturi od 32°C. Masna promena u našem istaživanju ustanovljena je samo u dva slučaja, najverovatnije zbog kratkog vremenskog perioda od davanja MDMA do žrtvovanja životinja. Drugi razlog je verovatno taj što su masne promene uglavnom opisivane kod ljudi umrlih usled trovanja *Ecstasy*-em, a oni koji uzimaju *Ecstasy* najčešće uzimaju i druge psihostimulanse koji su često u sastavu tableta, narkotike ili alkohol, pa masna promena može biti uzrokovana i tim agensima [14].

Ustanovljeno je da na temperaturama od 22 i 32°C dominira staza kod eksperimentalnih grupa, bez obzira na dozu MDMA, što je verovatno rezultat vazodilatacije kao odbrambene reakcije organizma na hipertemiju koja, uz znojenje, treba da omogući odavanje toplote. Na temperaturama od 22 i 32°C ustanovljena je i hemoragija. Umnožavanje Kupffer-ovih ćelija (fagocita) i kod kontrolnih i eksperi-

mentalnih grupa, na svim ispitivanim temperaturama najverovatnije je posledica manipulacije u toku uzimanja isečaka jetre, što se poklapa sa objavljenim podacima da manipulacija jetrom u toku transplantacije dovodi do aktivacije Kupfrer-ovih ćelija [15]. Anizocitoza i anizonukleoza javljaju se u sklopu hepatocelularne proliferacije kao odgovor na toksično oštećenje, a uočene su i retke mitotičke formacije.

Zaključak

Patohistološke promene jetre pacova u vidu portalne inflamacije, lobularne i periportne nekroze i krvarenja najizraženije su u grupi koja je dobila MDMA i boravila na temperaturi okoline od 32°C. Enzimski profil pokazuje da je na svim ispitivanim temperaturama okoline aktivnost AST viša kod eksperimentalnih grupa nego kod kontrolnih, ali je najizraženiji porast zabeležen na temperaturi od 32°C. Aktivnost ALT je viša kod eksperimentalnih nego kod kontrolne grupe na temperaturi od 32°C. *Ecstasy* je hepatotoksična supstanca, a patohistološki i biohemijski parametri pokazuju de se toksični efekti povećavaju sa rastom temperature okoline. Niža temperatura pokazala je zaštitni efekat od hepatoksičnosti MDMA kod pacova.

L I T E R A T U R A

1. *Karch S*. Karch's Pathology of Drug Abuse, 3rd edn. Boca Raton: CRC Press; 2002. p. 541.
2. *Turillazzi E, Riezzo I, Neri M, Bello S, Fineschi V*. MDMA toxicity and pathological consequences: a review about experimental data and autopsy findings. Curr Pharm Biotechnol 2010; 11(5): 500–9.
3. *Fawcett J, Busch KA*. Stimulants in psychiatry. In: *Schatzberg A, Nemeroff CB,* editors. The American Psychiatric Press Textbook of Psychopharmacology. 2nd ed. Washington, DC: American Psychiatric Press; 1998. p. 503–22.
4. *Melberg JE, Seiden LS*. Small changes in ambient temperature cause large changes in 3,4-methylendioxymethamphetamine(MDMA) - induced serotonin neurotoxicity and core body temperature and core body temperature in the rat. J Neurosci 1998; 18(13): 5086–94.
5. *Colado MI, Murray TK, Green AR*. 5-HT loss in rat brain following 3,4-methylendioxymethamphetamine (MDMA), p-chloroamphetamine and fenfluramine administration and effects of chlormethiazole and dizocipline. Br J Pharmacol 1993; 108(3): 583–9.
6. *Mathias R*. Methamphetamine brain damage in mice more extensive than previously thought. NIDA Notes 2000; 15(4): 1, 10.
7. *Brauer RB*. Liver transplantation for the treatment of fulminant hepatic failure induced by the ingestion of Ecstasy. Transplantation 1997; 10(3): 229–33.
8. *Andreu V, Mas A, Bruguera M, Salmerón JM, Moreno V, Nogué S,* et al. Ecstasy: a common cause of severe acute hepatotoxicity. J Hepatol 1998; 29(3): 394–7.
9. *Beitia G, Cobreros A, Sainz L, Cenarruzabeitia E*. Ecstasy-induced toxicity in rat liver. Liver 2000; 20(1): 8–15.
10. *Majkić Singh N*. Clinical ensymology. Subotica: AID Praktikum; 1993.
11. *Roques V, Perney P, Beaufort P, Hanslik B, Ramos J, Durand L*, et al. Acute hepatitis due to ecstasy. Presse Med 1998; 27(10): 468–70. (French)
12. *Schirren CA Berghaus TM, Sackmann M*. Thrombotic thrombocytopenic purpura after Ecstasy- induced acute liver failure. Ann Intern Med 1999; 130(2): 163.
13. *Aknine X, Gainville CSST, Ballanger HR, Bois AS*. Pertubed hepatis function in an ecstasy consumer. Presse Med 2004; 33(18 Suppl 1): 18–20.
14. *Baggott M, Heifets B, Jones RT, Mendelson J, Sferios E, Zehnder J*. Chemical analysis of ecstasy pills. JAMA 2000; 284(17): 2190.
15. *Schemmer P, Enomoto N, Bradford BU, Bunzendahl H, Raleigh JA, Lemasters JJ*, et al. Activated Kupffer cells cause a hypermetabolic state after gentle in situ manipulation of liver in rats. Am J Physiol Gastrointest Liver Physiol 2001; 280(6): G1076–82.

Primljen 29. XII 2009.
Revidiran 28. VII 2010.
Prihvaćen 8. IX 2010.

ORIGINAL ARTICLE

UDC:616-006::[616.6-006:616-008.854.6
DOI: 10.2298/VSP1107567D

Expression of regulatory proteins and proliferative activity in relation to phenotypic characteristics of upper urothelial carcinoma

Ekspresija regulatornih proteina i proliferativna aktivnost u vezi sa fenotipskim karakteristikama karcinoma gornjeg urotelijuma

Zana Dolićanin*, Ljubinka Janković Veličković[†], Biljana Djordjević[†], Milan Višnjić[‡], Ivana Pešić[§], Ana Ristić[†], Vesna Marjanović[‖]

***University of Priština-Kosovska Mitrovica, School of Medicine, Priština, Serbia; University of Niš, School of Medicine,** [†]Institute of Pathology, [‡]Clinic of Surgery, [§]Institute of Pathophysiology, **Niš, Serbia; [‖]Clinical Centre Niš,** Centre of Anaesthesiology and Reanimatology, **Niš, Serbia**

Abstract

Background/Aim. Deregulation of the normal cell cycle is common in upper urothelial carcinoma (UUC). The aim of this study was to investigate the expression of regulatory proteins of the cell cycle (p53, p16, cyclin D1, HER-2) and proliferative Ki-67 activity in UUC, and to determine their interaction and influence on the phenotypic characteristics of UUC. **Methods.** In 44 patients with UUC, histopathological and immunohistochemical analyses (p53, p16, cyclin D1, HER-2, and Ki-67) of tumors were done. **Results.** Overexpression/altered expression of p53, p16, cyclin D1 or HER-2 was detected in 20%, 57%, 64%, and 57% of tumors, respectively. Eleven (25%) UUC had a high proliferative Ki-67 index. Forty patients (91%) had at least one marker altered, while four (9%) tumors had a wild-type status. Analysis of relationship between expressions of molecular markers showed that only high expression of p53 was significantly associated with altered p16 activity ($p < 0.05$). High Ki-67 index was associated with the high stage ($p < 0.005$), solid growth ($p < 0.01$), high grade ($p < 0.05$), and multifocality $p < 0.05$) of UUC, while high expression of p53 was associated with the solid growth ($p < 0.05$). In regression models that included all molecular markers and phenotypic characteristics, only Ki-67 correlated with the growth ($p < 0.0001$), stage ($p < 0.01$), grade ($p < 0.05$) and multifocality ($p < 0.05$) of UUC; Ki-67 and HER-2 expression correlated with the lymphovascular invasion ($p < 0.05$). **Conclusions.** This investigation showed that only negative regulatory proteins of the cell cycle, p53 and p16, were significantly associated in UUC, while proliferative marker Ki-67 was in relation to the key phenotypic characteristics of UUC in the best way.

Key words:
urinary bladder; carcinoma; cell cycle proteins; ki-67 antigen.

Apstrakt

Uvod/Cilj. Deregulacija normalnog ćelijskog ciklusa je česta kod karcinoma gornjeg urotelijuma (KGU). Cilj ovog rada bio je da se ispita ekspresija regulatornih proteina ćelijskog ciklusa (p53, p16, ciklin D1, HER-2) i proliferativna aktivnost Ki-67 kod KGU i da se utvrdi njihov međusobni uticaj i uticaj na fenotipske karakteristike KGU. **Metode.** Kod 44 bolesnika sa KGU urađene su patohistološka i imunohistohemijska analiza (p53, p16, cyclin D1, HER-2 i Ki-67) tumora. **Rezultati.** Prekomerna ekspresija/izmenjena ekspresija p53, p16, ciklina D1 i HER-2 otkrivena je kod 20%, 57%, 64% i 57% tumora, redom. Jedanaest (25%) KGU imalo je visoki proliferativni Ki-67 indeks. Četrdeset bolesnika (91%) imalo je alteraciju najmanje jednog markera, dok su četiri (9%) tumora imala *wild-type* status. Analiza povezanosti ekspresije molekularnih markera pokazala je da je samo visoka ekspresija p53 bila značajno udružena sa izmenjenom p16 aktivnošću ($p < 0,05$). Visoki Ki-67 indeks bio je udružen sa visokim stadijumom ($p < 0,005$), solidnim rastom ($p < 0,01$), visokim gradusom ($p < 0,05$) i multifokalnošću ($p < 0,05$) KGU, dok je visoka ekspresija p53 bila udružena sa solidnim rastom ($p < 0,05$). U regresionom modelu koji je uključivao sve molekularne markere i fenotipske karakteristike, samo je Ki-67 korelisao sa rastom ($p < 0,0001$), stadijumom ($p < 0,01$), gradusom ($p < 0,05$) i multifokalnošću ($p < 0,05$) KGU, a ekspresija Ki-67 i HER-2 korelisala je sa limfovaskularnom invazijom ($p < 0,05$). **Zaključak.** Ovo istraživanje pokazalo je da su samo negativni regulatorni proteini ćelijskog ciklusa, p53 i p16, bili značajno povezani kod KGU, dok je proliferativni Ki-67 marker bio povezan sa ključnim fenotipskim karakteristikama KGU na najbolji način.

Ključne reči:
mokraćna bešika; karcinomi; ćelija, ciklus, proteini; ki-67 antigen.

Correspondence to: Ljubinka Janković Veličković, School of Medicine, Institute of Pathology, Bulevar Zorana Djindjića 81, 18 000 Niš, Serbia. Phone: +381 18 22 99 40, +381 64 22 84 789. E-mail: dravel@open.telekom.rs

Introduction

A normal cell has three different states: static, division, or apoptosis. Most urothelial cells are not dividing under physiological condition [1, 2]. Many proteins act during the process of DNA replication, but also in cell grown regulation. Defects in the regulation of the DNA replication can lead to cancer. Activated cell proliferation may produce the loss of cell cycle arrest due to encoding of cellular regulatory genes or deactivation of tumor suppressor genes [3–6].

Deregulation of the normal cell cycle is common in upper urothelial carcinoma (UUC). Mutations in the p53 gene were frequently found in invasive UUC as well as in high-grade superficial UUC, while these mutations were rare in well-differentiated superficial UUC. p53 mutations result in prolonged half-life and accumulation of the p53 protein to a level that makes it detectable immunocytochemically (ICH) in tumor cell nuclei. In contrast, the wild-type p53 protein has a short half-life, measured as only 6-30 minutes [7–11].

Also, a new family of negative cell cycle regulators, the cyclin-dependent kinase inhibitor gene INK4 and KIP, has been identified. $p16^{INK4A}$, as members of the INK4, and KIP family proteins ($p21^{CIP1}$, $p27^{KIP1}$, and $p57^{KIP2}$) are inhibitors of cyclin-dependent kinases (CDKs). $p16^{INK4A}$ increases the Rb protein level as a control of the G1-S cell cycle checkpoint, and inhibits the CDK4/6 kinase. The loss of p16 function leads to the loss of pRb function and inappropriate cell cycling [12, 13]. Some findings explain the importance of inactivation through the $p16^{INK4A}$ pathway during the oxidative stress that leads to the activation of transitional cell carcinoma (TCC) [14].

Cyclin D1 is an important positive regulator of the G1–S transition, and expressed in the early G1 in response to mitogenic signals. Its expression is maximal in the middle G1 phase. Cyclin D contributes to regulate G1 progression by forming a complex with different CDK catalytic component [15, 16]. Cyclin D1 gene (CCND1) is located at chromosome 11q13, and amplification of the chromosomal region is frequently detected in TCCs of the bladder. CCND1 overexpression occurs in TCCs of the bladder and may be associated with growth of low-grade papillary tumors [17].

The proto-oncogene HER-2 (located on 17q21) encodes tyrosine kinase growth factor receptor and regulates cell growth and differentiation. The amplification of HER-2 or overexpression of its product is associated with malignant cell transformation and a poor prognosis in prostate, bladder and breast tumors. In urothelial carcinomas, it has been associated with tumor grade, tumor stage, and a patient's outcome [18]. HER2 positive tumors were associated with greater metastatic potential [19].

Ki-67, a marker of cellular proliferation, is nuclear protein complex expressed in the G1, S, G2 and M phases of the cell cycle of proliferating cells. Ki-67 may be involved in ribosome biosynthesis during cell proliferation, suggesting that the Ki-67 antigen is not directly involved in the cell cycle regulation [20].

The aim of this study was to investigate the expression of regulatory proteins of the cell cycle (p53, p16, cyclin D1, HER-2) and proliferative Ki-67 activity in UUC, and to determine their interaction and influence to the phenotypic characteristics of UUC.

Methods

The investigation included 44 consecutive patients with UUC. The analysis was done on 37 pelvic and 7 ureteral tumors. The patients included in the study had undergone nephroureterectomy with removal of bladder cuff. Extended lymphadenectomy was not routinely performed. All the cases of UUC were diagnosed at the Institute of Pathology, School of Medicine, Niš. The histological sections were processed from tissue fixed in 10% formalin by standard techniques, and stained with haematoxylin and eosin (H&E). H&E-stained slides were used to assess histological grade (low and high grade) [21], pathologic stage (pT) [22], growth of tumor (papillary/solid), multifocality, and lymphovascular invasion (LVI). Authors compared low stage non-muscle invasive tumor (pTa-pT1) and high stage muscle invasive (pT2-pT4) tumor [23].

Immunohistochemistry and scoring

We performed p53, p16, cyclin D1, HER-2, and Ki-67 immunohistochemical staining using serial sections from the same paraffin-embedded blocks. Tumors were analyzed by using the mouse monoclonal antibody and a standard avidin-biotin immunoperoxidase complexes detection system, according to the manufacturer's protocol (Dako LSAB2R system-HRP). Staining and scoring protocols for p53, p16, cyclin D1, HER-2, and Ki-67 are given in Table 1.

Table 1

Immunostaining protocol

Antibody	Clone / Source	Dilution	Expression
p53	Pab 1801, IgG1 / Newcastle	1:50	nuclear
p16	Clone 6H12, IgG2b / Newcastle	1:40	nuclear
Cyclin D1	P2D11F11, IgG2a / Newcastle	Ready to use	nuclear
HER-2	Code A 0485 / Dako	1:300	cell membrane
Ki-67	MIB-1, Izotip IgG1, kappa / Dako	1:100	nuclear

Before quantifying the immunohistochemical results, the technique quality was assessed and those areas with greater positivity were selected, avoiding peripheral area measurement, necrosis or artifact.

Nuclear p53 immunoreactivity was considered altered when samples demonstrated at least 10% nuclear reactivity, as it has been shown that accumulation of p53 protein in 10% or more of the tumor cell nuclei strongly correlates with

mutations in the p53 gene [24]. The staining protocol for p16 nuclear protein includes heterogeneous, homogeneous and negative findings. Tumor was considered to have a normal heterogeneous p16 pattern if it had relatively weak nuclear staining with considerable differences in nuclear intensity, including many negative cells. Strong 16 staining considered if the majority of the malignant cells had intensive p16 nuclear expression and p16 negative tumor cells were rare. Tumor was termed p16 negative if no malignant cells had positive staining. Tumor without or with overexpression of p16 was categorized as altered [25]. The cutoff value for cyclin D1 in the tumor tissue was set at the level of expression in the normal tissue (5%). For testing HER-2 (C-erbB2) status we used the HercepTest scoring system devised by DAKO. HER-2 cell membrane specific immunoreactivity were scored by estimating the percentage of positive tumor cell as follows: score 0, no immunoreactive cells; score +1, positivity in < 5% cancer cells; score +2, positivity in 5–50% cancer cells; and score +3, positivity in > 50% of cancer cells. The specimens were considered positive for HER-2 expression when the score was +2 or +3. The nuclei in morphologically malignant cells were considered positive for Ki-67 antigen when they showed dark brown granular staining. Stromal or peritumoral lymphocytes were present in most cases and served as internal controls for Ki-67. The cutoff value for Ki-67 in the tumor tissue was 10% [25, 26].

Slides were reviewed independently by the three investigators. Interobserver discrepancies were resolved using a double headed microscope. Only nuclear expression was recorded for p53, p16, cyclin D1, and Ki-67. The number of distinctly positive tumor cell nuclei was counted under high power (× 400) using a 10 × 10 eyepiece grid. In total, 1,000 tumor cells were assessed. The number of positive nuclei was expressed as a percentage of all tumor cell nuclei counted.

Statistical analysis

For the purposes of statistical analysis, pathological tumor stage (low *vs* high), grade (low *vs* high), growth pattern (papillar *vs* solid), LVI (yes *vs* no), and multifocality (yes *vs* no) were evaluated as dichotomized variables. The Fisher's exact test and the χ^2 test were used to evaluate the association of p53, p16, cyclin D1, HER-2, Ki-67 with phenotypic characteristics of tumors (stage, grade, growth, lymphovascular invasion), and multifocality. To determinate influence of regulatory proteins (p53, p16, cyclin D1, HER-2, Ki-67) on conventional pathological parameters regression analysis was performed.

All analyses were performed with the SPSS statistical package (SPSS version 10.0 for Windows). The result was considered statistically significant if $p < 0.05$.

Results

Clinical and pathological features

The age of 44 patients with UUC ranged from 22 to 87 years, with a mean age of 63.5 years. There was a male predominance (29 patients) with relation M : F = 2 : 1.

The number of patients with low grade and high grade UUC was 27 (61%), and 17 (39%), respectively. The pathological stage was low in 15 (34%), and high stage in 29 (66%) patients. Tumors had papillary growth in 24 (55%), and solid in 20 (45%) specimens. Also, LVI and multifocality were detected in 12 (27%), and 9 (20%) UUC, respectively.

There was a significant association between pathological stage and grade, as well as pathological stage and LVI ($\chi^2 = 6.15$, $p < 0.05$, and $\chi^2 = 8.53$, $p < 0.005$, respectively). Growth pattern and multifocality of UUC were not in significant association with the pathological stage (Table 2).

Evaluation of immunohistochemical staining

The normal urothelium of the kidney pelvis and ureter showed heterogeneous expression of p16 (wild type) and the absence of p53 (wild type).

Table 2

Morphological finding and pathological stage

Morphological finding	Pathological stage		*p*
	low stage n (%)	high stage n (%)	
Grade			
low grade	13 (30)	14 (32)	< 0.05
high grade	2 (4)	15 (34)	
Growth			
papillary	11 (25)	13 (30)	N.S.
solid	4 (9)	16 (36)	
LVI			
yes	0 (0)	12 (27)	< 0.005
no	15 (34)	17 (39)	
Multifocality			
yes	3 (7)	6 (14)	N.S.
no	12 (27)	23 (52)	

LVI – lymphovascular invasion; N.S. – no significance

Tumor cells reacted positively for p53, p16, cyclin D1 (Figure 1, a–c), and Ki-67 were predominantly stained in the nucleus, while HER-2 had expression on the cell membrane (Figure 1d).

The mean p53 labeling index was 6.88 ± 15.67 (range, 0–89.2) and 18 specimens (41%) were positive for p53 ex-

pression. Expression of p53 was low in 35 (80%) and high in 9 (20%) specimens (Fig.1a). Immunohistochemical analysis of p16 showed that 25 (57%) tumors had altered p16 function, i.e. 16 (36%) UUC were p16 negative, while 9 (21%) tumors had strong, homogeneous nuclear staining. Cytoplasm p16 staining was common in the presence of nuclear staining, particularly in those tumors with the strong p16 expression (Figure 1b), but was not found in p16 negative tumors. A normal heterogeneous p16 staining pattern was detected in 19 (43%) UUC. The mean labeling index for cyclin D1 was 11.91 ± 11.63 (range, 0–41.23), and 30 specimens (68%) were positive for cyclin D1 expression. Expression of cyclin D1 was low in 16 (36%) and high in 28 (64%) specimens (Figure 1c). Investigation of the HER-2 status of 44 UUC showed that 25 (57%) tumors was HER-2 positive (≥ 2+ staining), while only 9 (20%) of them were classified as 3+ positive (Figure 1d). The mean Ki-67 labeling index was 10.49 ± 9.00 (range, 0.1–34.3). Expression of Ki-67 was low in 33 (75%) and high in 11 (25%) specimens (Figures 1, e and f).

Number of altered cell cycle regulatory proteins in UUC

Forty patients (91%) had at least one marker altered, while four (9%) tumors had wild-type status (p53, p16, cyclin D1, HER-2). The majority (64%) of UUC had alteration of one or two markers: 12 (27%) one and 16 (37%) two markers. Alterations in the three or four cell cycle regulatory proteins occurred in twelve (27%) patients with UUC, in 5 (11%) patients three and in 7 (16%) patients four markers (Table 3). An analysis of the relationship between expression of molecular markers showed that only high expression of p53 was significantly associated with altered p16 activity ($\chi^2 = 4.79$, $p < 0.05$) (data shown in Table 4 are only for markers which reached statistical significance).

Correlation of immunohistochemical staining with conventional pathological features

Tables 5 and 6 show the relationships between conventional pathological parameters and immunohistochemical

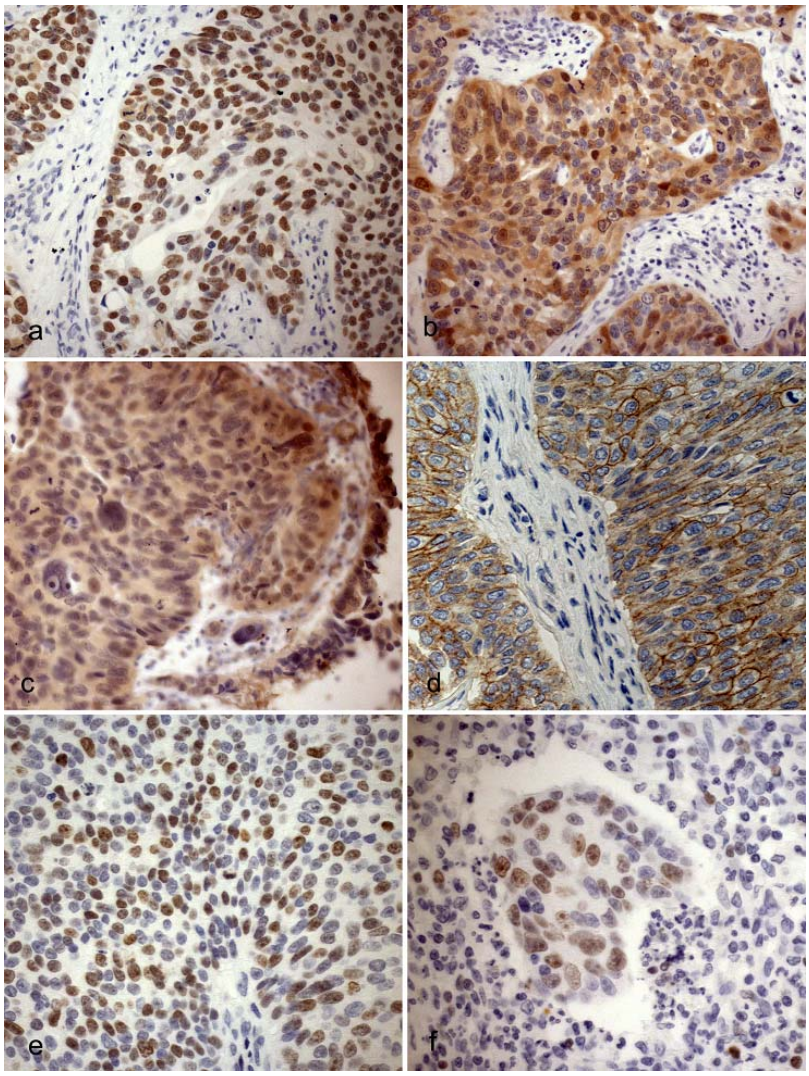


Fig. 1 – The representative positive staining of regulatory proteins in upper urothelial carcinoma (UUC), with strong nuclear p53 activity in solid growth of tumor (a), homogeneous p16 nuclear and cytoplasm expression (b), nuclear cyclin D1 staining in high grade tumor (c); HER-2 positive tumor cells classified as 3+ positive (d); high proliferative Ki-67 activity in solid growth (e), and LV invasion (f) (ABC, original magnification × 400)

Table 3

Alteration of cell cycle regulatory proteins in upper urothelial carcinoma (UUC)

One marker	n	Two markers	n	Three markers	n	Four markers	n
Cyclin D1	5	Cyclin D1+ HER2	6	Cyclin D1+ p16+ HER2	5	Cyclin D1+ p16+p53+HER2	7
p16	4	Cyclin D1+p16	4				
HER 2	3	Cyclin D1+p53	1				
		p16+ HER2	4				
		p16+p53	1				
Total	12 (27%)		16 (37%)		5 (11%)		7 (16%)

Note: 4 (9%) UUC have wild type of molecular markers

Table 4

Association of p53 and p16 in upper urothelial carcinoma (UUC)

Marker	p53		*p*
	low	high	
p16 normal	18	1	< 0.05
p16 altered	17	8	

Table 5

Ki-67, p53 and cyclin D1 index in relation to grade, stage, growth, lymphovascular invasion (LVI) and multifocality

Fenotipic characteristics	Total (n = 44)	Ki-67		*p*	p53		*p*	CyclinD1		*p*
		low	high		low	high		low	high	
Grade										
low grade	27	23	4	< 0.05	23	4	N.S.	9	18	N.S.
high grade	17	10	7		12	5		7	10	
Stage										
low stage	15	15	0	< 0.005	13	2	N.S.	5	10	N.S.
high stage	29	18	11		22	7		11	18	
Growth										
papillary	24	22	2	< 0.01	22	2	< 0.05	10	14	N.S.
solid	20	11	9		13	7		6	14	
LVI										
yes	12	7	5	N.S.	8	4	N.S.	4	8	N.S.
no	32	26	6		27	5		12	20	
Multifocality										
yes	9	4	5	< 0.05	8	1	N.S.	2	7	N.S.
no	35	29	6		27	8		14	21	

N.S. –no significance

Table 6

p16 and HER-2 expression in relation to grade, stage, growth, lymphovascular invasion (LVI) and multifocality

Fenotipic characteristics	Total (n = 44)	p16 altered n (%)	*p*	HER-2 score ≥ 2 n (%)	*p*
Grade					
low grade	27	13 (30)	N.S.	17 (39)	N.S.
high grade	17	12 (27)		8 (18)	
Stage					
low stage	15	9 (20)	N.S.	10 (23)	N.S.
high stage	29	16 (36)		15 (34)	
Growth					
papillary	24	15 (34)	N.S.	14 (32)	N.S.
solid	20	10 (23)		11 (25)	
LVI					
yes	12	8 (18)	N.S.	4 (9)	N.S.
no	32	17 (39)		21 (48)	
Multifocality					
yes	9	5 (11)	N.S.	6 (14)	N.S.
no	35	20 (45)		19 (43)	

N.S. – no significance

staining of Ki-67, p53, cyclin D1, p16 and HER-2 in UUC. The Ki-67 labeling index significantly correlated with the grade ($\chi^2 = 3.87$, $p < 0.05$), stage (Fisher = 0.0045, $p < 0.005$), growth ($\chi^2 = 7.82$, $p < 0.01$) and multifocality of tumor ($\chi^2 = 5.63$, $p < 0.05$). The p53 labeling index correlated only with the growth pattern (Fisher = 0.0031, $p < 0.05$). Expression of p16, cyclin D1, and HER-2 was not significantly associated with the conventional pathological parameters and multifocality of tumor. Also, tumors which showed HER-2 score 3 were more often in high than in low stage [7/29 (24%) *vs* 2/15 (13%)], while strong expression of cyclin D1 (> 25%) had a similar distribution in dependence of the stage [5/29 (17%) *vs* 3/15 (20%)].

A possible correlation between molecular markers (p53, p16, cyclin D1, HER-2, Ki-67) and standard pathological features was investigated. Regression analysis showed that proliferative marker Ki-67 correlated with the growth ($p < 0.0001$), stage ($p < 0.001$), grade ($p < 0.05$), and multifocality ($p < 0.05$) of UUC on the best way. Ki-67 and HER-2 expression correlated with the lymphovascular invasion ($p < 0.05$) (Table 7).

Also, we found that the expression of cell cycle regulators is commonly altered in UUC patients, with 91% of patients having altered expression of at least one marker, and with 16% exhibiting altered expression of p53 and p16. The association of p53 and p16 tumor suppressor pathway was significant in UUC.

P16 is specific CDK inhibitor, and negatively regulates cell-cycle progression in a p53-independent manner [4].

Shariat et al. [24] have found alteration of at least one of the three tumor markers in 83% of patients, and alteration of p53, p21, and pRB/p16 in 26% of patients with bladder cancer. Also, the authors have found that tumors with alterations of p53 and pRB/p16, were associated with muscle-invasive disease. They had significantly higher risk for unfavorable clinical outcome than patients with only one of the two markers altered.

The role of cell cycle regulators in bladder cancer progression seems to be a complex accumulation of genetic alteration, from which p53 and p16 seem to be associated with the later stages of bladder cancer clinical progression [24].

Table 7

Influence of Ki-67 and HER-2 on fenotypic characteristics of tumor

Constant	Dependent variable	Standardized coefficients β	t	*p*
Ki-67	Grade	0.309	2.061	< 0.05
Ki-67	Stage	0.448	3.054	< 0.01
Ki-67	Growth	0.596	4.606	< 0.0001
Ki-67	Multifocality	0.387	2.622	< 0.05
Ki-67	LVI	0.364	2.621	< 0.05
HER-2	LVI	-0.381	-2.522	< 0.05

LVI – lymphovascular invasion

Discussion

Various histopathological and clinical parameters are known to have prognostic significance in UUC. These parameters include tumor stage, histological grade, multicentricity, tumor growth pattern (papillary *vs* solid), LVI, and tumor cell proliferation. While histopathological criteria can provide important morphological information about tumors, they are not reliable to specify the risk for progression or response to treatment for a patient with UUC [27, 28].

The mutations of tumor suppressor gene p53 of chromosome 17 have already been reported in urothelial carcinoma. Several studies have demonstrated a positive correlation between p53 abnormalities and higher tumor stage [7, 29]. Esring et al. [30] observed that p53 overexpression correlated significantly with recurrence and crude survival, and in multivariate regression analysis, it was a factor independent of pathological stage and histological grade.

This study showed that overexpression of p53 was detected in 20% of tumors. UUC with high grade, high stage, solid growth and LVI had more common overexpression of p53, however statistically significant only in solid tumors. The sample size may have limit of our ability to detect other differences. Our finding is similar to investigation of Kamijima et al. [9].

In this study, nuclear expression of cyclin D1 in more than 5% of tumor cells was found in 64% of the cases and was not associated with the growth, differentiation and stage. Very strong expression of cyclin D1 (> 25%) was detected in 18% of UUC.

Cyclin D1 overexpression may be an important early event in the progression of TCCs. Its association with well-differentiated, papillary tumors suggests that cyclin D1 may also play a role in tumor differentiation [31, 32]. Khan et al. [31] suggested that a cyclin D1 dependent pathway determines the evolution of a group of well-differentiated low-stage papillary TCCs, whereas tumors that evolve *via* cyclin D1 independent mechanisms are less differentiated and pathologically more aggressive. These observations are consistent with the reported accumulation of Rb and p53 mutations in advanced TCCs [33].

Deregulation of HER-2 was present in 57% of UUC, where 20% of those tumors had HER-2 score 3 and more often in high stage of the disease. No significant association was present with the expression of HER-2 and other conventional pathological parameters.

The level of expression and the prognostic significance of HER-2 protein in urothelial cancer varies among different studies, with some revealing no prognostic relevance and other suggesting a better or worse prognosis [34, 36].

Strong HER-2 overexpression was detected using immunohistochemistry in 23% of bladder urothelial cancer, and in 33% of patients with metastases. HER-2 overexpression is strongly associated (95%) with gene amplification assessed using FISH [33].

Inoue et al. [18] did not found a correlation between the relative increase in a HER-2 copy number and tumor stage. HER-2 amplification, found more frequently in pT2-T4 tumors than in pTa-T1, did not correlate with tumor grade. The relative increase of the HER-2 copy number may be associated with the number of recurrences and the presence of CIS.

In our study, high proliferative Ki-67 index had 25% of UUC. Overexpression of Ki-67 was significantly associated with the advanced pathological stage, higher tumor grade, solid growth and multifocality of UUC. The regression analysis showed that proliferative marker Ki-67 correlated with phenotypic characteristics of UUC in the best way. Also, Ki-67 activity and HER-2 oncogene correlated with the lymphovascular invasion.

Proliferative marker Ki-67 has a prognostic value in renal cell carcinoma and urothelial neoplasms of the urinary bladder [37], being associated with tumor grade, stage, recurrence and prognosis of urothelial carcinoma [9, 38–40], as well as with LVI, and metastases to lymph nodes. Ki-67 is the best predictor of recurrence in noninvasive bladder tumors, but also is an independent predictor of disease recurrence, progression, and response to intravesical therapy in patients with nonmuscle-invasive bladder cancer. Its high expression is related to a poor survival. The availability of Ki-67 and MIB1 antibodies in most laboratories makes it an ideal marker to be used in the daily evaluation of urothelial tumors [9, 12, 41].

Conclusion

This investigation showed that only negative regulatory proteins of the cell cycle, p53 and p16, were significantly associated in UUC, while the proliferative marker Ki-67 was in relation to the key phenotypic characteristics of UUC in the best way.

Acknowledgments

This work was supported by the grant No 175092 from the Ministry of Science and Technological Development of the Republic of Serbia.

R E F E R E N C E S

1. *Garcia-España A, Salazar E, Sun TT, Wu XR, Pellicer A.* Differential expression of cell cycle regulators in phenotypic variants of transgenically induced bladder tumors: implications for tumor behavior. Cancer Res 2005; 65(4): 1150–7.
2. *Margulis V, Shariat SF, Ashfaq R, Sagalowsky AI, Lotan Y.* Ki-67 is an independent predictor of bladder cancer outcome in patients treated with radical cystectomy for organ-confined disease. Clin Cancer Res 2006; 12(24): 7369–73.
3. *Denzinger S, Mohren K, Knuechel R, Wild PJ, Burger M, Wieland WF,* et al. Improved clonality analysis of multifocal bladder tumors by combination of histopathologic organ mapping, loss of heterozygosity, fluorescence in situ hybridization, and p53 analyses. Hum Pathol 2006; 37(2): 143–51.
4. *Keegan PE, Lunec J, Neal DE.* p53 and p53-regulated genes in bladder cancer. Br J Urol 1998; 82(5): 710–20.
5. *Koed K, Wiuf C, Christensen LL, Wikman FP, Zieger K, Møller K,* et al. High-density single nucleotide polymorphism array defines novel stage and location-dependent allelic imbalances in human bladder tumors. Cancer Res 2005; 65(1): 34–45.
6. *Nishikawa T, Salim EI, Morimura K, Kaneko M, Ogawa M, Kinoshita A,* et al. High susceptibility of p53 knockout mice to esophageal and urinary bladder carcinogenesis induced by N, N-dibutylnitrosamine. Cancer Lett 2003; 194(1): 45–54.
7. *Ehdaie B, Theodorescu D.* Molecular markers in transitional cell carcinoma of the bladder: New insights into mechanisms and prognosis. Indian J Urol 2008; 24(1): 61–7.
8. *Gonzalez-Campora R, Davalos-Casanova G, Beato-Moreno A, Garcia-Escudero A, Pareja Megia MJ, Montironi R,* et al. BCL-2, TP53 and BAX protein expression in superficial urothelial bladder carcinoma. Cancer Lett 2007; 250(2): 292–9.
9. *Kamijima S, Tobe T, Suyama T, Ueda T, Igarashi T, Ichikawa T,* et al. The prognostic value of p53, Ki-67 and matrix metalloproteinases MMP-2 and MMP-9 in transitional cell carcinoma of the renal pelvis and ureter. Int J Urol 2005; 12(11): 941–7.
10. *Mhawech-Fauceglia P, Cheney RT, Fischer G, Beck A, Herrmann FR.* FGFR3 and p53 protein expressions in patients with pTa and pT1 urothelial bladder cancer. Eur J Surg Oncol 2006; 32(2): 231–7.
11. *van Rhijn BW, van der Kwast TH, Vis AN, Kirkels WJ, Boevé ER, Jöbsis AC,* et al. FGFR3 and P53 characterize alternative genetic pathways in the pathogenesis of urothelial cell carcinoma. Cancer Res 2004; 64(6): 1911–4.
12. *Aaltonen V, Koivunen J, Laato M, Peltonen J.* Heterogeneity of cellular proliferation within transitional cell carcinoma: correlation of protein kinase C alpha/betaI expression and activity. J Histochem Cytochem 2006; 54(7): 795–806.
13. *Weebadda WK, Jackson TJ, Lin AW.* Expression of p16INK4A variants in senescent human fibroblasts independent of protein phosphorylation. J Cell Biochem 2005; 94(6): 1135–47.
14. *Hori Y, Oda Y, Kiyoshima K, Yamada Y, Nakashima Y, Naito S,* et al. Oxidative stress and DNA hypermethylation status in renal cell carcinoma arising in patients on dialysis. J Pathol 2007; 212(2): 218–26.
15. *Fu TY, Tu MS, Tseng HH, Wang JS.* Overexpression of p27kip1 in urinary bladder urothelial carcinoma. Int J Urol 2007; 14(12): 1084–7.
16. *Lee CC, Yamamoto S, Morimura K, Wanibuchi H, Nishisaka N, Ikemoto S,* et al. Significance of cyclin D1 overexpression in transitional cell carcinomas of the urinary bladder and its correlation with histopathologic features. Cancer 1997; 79(4): 780–9.
17. *Wang L, Habuchi T, Takahashi T, Mitsumori K, Kamoto T, Kakehi Y,* et al. Cyclin D1 gene polymorphism is associated with an increased risk of urinary bladder cancer. Carcinogenesis 2002; 23(2): 257–64.
18. *Inoue T, Sato K, Tsuchiya N, Matsuura S, Iinuma M, Habuchi T,* et al. Numeric aberrations of HER-2 and chromosome 17 detected by fluorescence in situ hybridization in urine-exfoliated cells from patients with urothelial carcinoma. Urology 2004; 64(3): 617–21.
19. *Black PC, Dinney CP.* Growth factors and receptors as prognostic markers in urothelial carcinoma. Curr Urol Rep 2008; 9(1): 55–61.

20. *Semple JW, Duncker BP.* ORC-associated replication factors as biomarkers for cancer. Biotechnol Adv 2004; 22(8): 621–31.
21. *Lopez-Beltran A, Sauter G, Gasser T, Hartmann A, Schmitz-Drager BJ, Helpap B, et al.* Tumors of the urinary system. Infiltrating urothelial carcinoma. In: *Eble JN, Sauter G, Epstein JI, Sesterhenn IA*, editors. World Health Organization classification of tumours. Pathology and genetics. Tumours of the urinary system and male genital organs. Lyon: IARC; 2004. p. 93–109.
22. *Sobin LH, Wittekind C.* TNM classification of malignant tumors, 6th ed. New York: Wiley-Liss; 2002.
23. *Genega EM, Kapali M, Torres-Quinones M, Huang WC, Knauss JS, Wang LP,* et al. Impact of the 1998 World Health Organization/International Society of Urological Pathology classification system for urothelial neoplasms of the kidney. Mod Pathol 2005; 18(1): 11–8.
24. *Shariat SF, Tokunaga H, Zhou J, Kim J, Ayala GE, Benedict WF,* et al. p53, p21, pRB, and p16 expression predict clinical outcome in cystectomy with bladder cancer. J Clin Oncol 2004; 22(6): 1014–24.
25. *Benedict WF, Lerner SP, Zhou J, Shen X, Tokunaga H, Czerniak B.* Level of retinoblastoma protein expression correlates with p16 (MTS-1/INK4A/CDKN2) status in bladder cancer. Oncogene 1999; 18(5): 1197–203.
26. *Migaldi M, Rossi G, Maiorana A, Sartori G, Ferrari P, De Gaetani C,* et al. Superficial papillary urothelial carcinomas in young and elderly patients: a comparative study. BJU Int 2004; 94(3): 311–6.
27. *Leppert JT, Shvarts O, Kawaoka K, Lieberman R, Belldegrun AS, Pantuck AJ.* Prevention of bladder cancer: a review. Eur Urol 2006; 49(2): 226–34.
28. *Novara G, De Marco V, Gottardo F, Dalpiaz O, Bouygues V, Galfano A,* et al. Independent predictors of cancer-specific survival in transitional cell carcinoma of the upper urinary tract: multiinstitutional dataset from 3 European centers. Cancer 2007; 110(8): 1715–22.
29. *Soriano P, Navarro S, Gil M, Llombart-Bosch A.* Small-cell carcinoma of the urinary bladder. A clinico-pathological study of ten cases. Virchows Arch 2004; 445(3): 292–7.
30. *Esrig D, Elmajian D, Groshen S, Freeman JA, Stein JP, Chen SC*, et al. Accumulation of nuclear p53 and tumor progression in bladder cancer. N Engl J Med 1994; 331(19): 1259–64.
31. *Khan AA, Abel PD, Chaudhary KS, Gulzar Z, Stamp GW, Lalani EN.* Inverse correlation between high level expression of cyclin E and proliferation index in transitional cell carcinoma of the bladder. Mol Pathol 2003; 56(6): 353–61.
32. *Schulz WA.* Understanding urothelial carcinoma through cancer pathways. Int J Cancer 2006; 119(7): 1513–8.
33. *Cordon-Cardo C, Zhang ZF, Dalbagni G, Drobnjak M, Charytonowicz E, Hu SX,* et al. Cooperative effects of p53 and pRB alterations in primary superficial bladder tumors. Cancer Res 1997; 57(7): 1217–21.
34. *Habuchi T, Marberger M, Droller MJ, Hemstreet GP 3rd, Grossman HB, Schalken JA*, et al. Prognostic markers for bladder cancer: International Consensus Panel on bladder tumor markers. Urology 2005; 66(6 Suppl 1): 64–74.
35. *Jimenez RE, Hussain M, Bianco FJ Jr, Vaishampayan U, Tabazcka P, Sakr WA,* et al. Her-2/neu overexpression in muscle-invasive urothelial carcinoma of the bladder: prognostic significance and comparative analysis in primary and metastatic tumors. Clin Cancer Res 2001; 7(8): 2440–7.
36. *de Pinieux G, Colin D, Vincent-Salomon A, Couturier J, Amsellem-Ouazana D, Beuzeboc P*, et al. Confrontation of immunohistochemistry and fluorescent in situ hybridization for the assessment of HER-2/ neu (c-erbb-2) status in urothelial carcinoma. Virchows Arch 2004; 444(5): 415–9.
37. *El-Kott AF.* Flow cytometry and Ki-67 expression in rat's urinary bladder carcinogenesis treated with Allium Sativum. Cancer Ther 2007; 5: 185–92.
38. *Fromont G, Rouprêt M, Amira N, Sibony M, Vallancien G, Validire P,* et al. Tissue microarray analysis of the prognostic value of E-cadherin, Ki67, p53, p27, survivin and MSH2 expression in upper urinary tract transitional cell carcinoma. Eur Urol 2005; 48(5): 764–70.
39. *Kunju LP, Lee CT, Montie J, Shah RB.* Utility of cytokeratin 20 and Ki-67 as markers of urothelial dysplasia. Pathol Int 2005; 55(5): 248–54.
40. *Shamsdin SA, Mehrabani D, Hosseinzadeh M.* Expression of Ki-67, C-erbB2 and EGFR in TCC of the urinary bladder and their correlation with tumor grading. Iranian Red Cresc Med J 2008; 10(2): 95–9.
41. *Quintero A, Alvarez-Kindelan J, Luque RJ, Gonzalez-Campora R, Requena MJ, Montironi R,* et al. Ki-67 MIB1 labelling index and the prognosis of primary TaT1 urothelial cell carcinoma of the bladder. J Clin Pathol 2006; 59(1): 83–8.

Received on March 25, 2010.
Accepted on October 4, 2010.

ORIGINAL ARTICLE

UDC: 611.13/.16::611.98]:617-089.844
DOI: 10.2298/VSP1107575G

Skin vascularisation field by the ascending branch of the peroneal artery *ramus perforans*

Zona vaskularizacije kože ascedentnom granom *ramus*-a *perforans*-a peronealne arterije

Milomir Gačević*, Milan Milisavljević†, Marijan Novaković*, Danilo Vojvodić#, Ivica Milosavljević‡ , Milena Jović‡, Boban Đorđević*, Žarko Borović§, Nikola Ostojić*, Mikica Lalković*, Saša Milićević*

Military Medical Academy, *Clinic for Plastic Surgery and Burns, #Medical Research Institute, ‡Institute of Pathology and Forensic Medicine, **Belgrade, Serbia; †University of Belgrade, School of Medicine, Anatomy Institute, Belgrade, Serbia; §Clinical Center, Podgorica, Montenegro**

Abstract

Background/Aim. Soft tissue defects in the distal third of the lower leg are persistent and constitute a major problem in the reconstructive surgery. This study presents an analysis of the anatomical vascularization filed of ascending branch of the peroneal artery *ramus perforans* (PARS). The aim of this study was to assess reliability of the distal flap on the antero-lateral aspect of a lower leg distal third. **Methods.** Direct gentiana violet injection into the interosseal perforator of ten fresh cadaveric lower legs with subsequent corrosion acrylic preparation was performed to reveal vascularization filed of the ascending branch of the PARP. Height, length, diameter and communication of perforating branch and its subsequent smaller ascending and descending branches were determined. The CAMIA software was used. **Results.** Our results show that the PARP is always present. Its origin from the peroneal artery is at the medial height of 66 mm when measured from the inferior border of the lateral malleolus. Medium length of *ramus perforans* is 51.7mm. After transition through the interosseous membrane, *ramus perforans* divides into ascending and descending branches. The diameter proximal to the level of bifurcation is 1.37 mm (variation 1.0–1.8 mm), and the diameter of the ascending branch distal to the level of bifurcation is 1 mm. Using CAMIA software, the medium length, width and area of the vascularization filed labeled with gentian violet were calculated to be 164 mm (variation 125–210 mm), 66 mm (57–77 mm), and 10,305 mm^2 (6,385 mm^2–14,341 mm^2), respectively. C**onclusion.** Our results support the use of fasciocutaneous distal flap, vascularized by the ascending branch of the PARP for reconstruction of soft tissue defects in the distal third of the lower limb, malleolar regions and *dorsum.*

Key words:
leg; soft tissue injuries; therapeutics; reconstructive surgical procedures; surgical flaps; anatomy, regional.

Apstrakt

Uvod/Cilj. Mekotkivni defekti distalne trećine potkolenice i dalje predstavljaju veliki problem u rekonstruktivnoj hirurgiji. Cilj ove studije bio je da se ispita anatomsko polje vaskularizacije ascedentne grane *ramus*-a *perforans*-a peronealne arterije (RPPA) radi provere mogućnosti kreiranja distalno baziranog režnja na anterolateralnoj strani donje trećine potkolenice. **Metode.** Na deset svežih kadaveričnih potkolenica koristili smo metod ubrizgavanja boje gencijana violet direktno u interosalni perforator. Na ovaj način dobili smo polje vaskularizacije ascedentne grane RPPA. Metodom izrade korozivnih akrilatnih preparata kod deset kadaveričnih potkolenica odredili smo visinu, dužinu, promer i komunikacije RPPA i njegove ascedentne grane. **Rezultati.** RPPA je konstantan krvni sud (prosečna dužina 51,7 mm). Odvaja se od peronealne arterije kroz interosealnu membranu na prosečnoj visini od 66 mm, mereno od donje ivice lateralnog maleolusa. Po prolasku kroz interosealnu membranu deli se na ascedentnu i descedentnu granu. Promer *ramus*-a pre bufurkacije iznosi prosečno 1,37 mm (1–1,8 mm), a ascedentne grane na početku 1 mm (0,8–1,3 mm). Prosečna vrednost dužine dobijene prebojenosti na ascedentnoj grani i zone vaskularizacije potencijalnog režnja je 164 mm (125–210 mm), širine 66 m (57–77), a površine 10 305 mm^2 (6 385–14 341 mm^2), određeno pomoću softvera za automatsku obradu slike CAMIA. **Zaključak.** Svi dobijeni parametri ukazuju na dobru i konstantnu vaskularizaciju opisanog segmenta kože od ascedentne grane RPPA i na mogućnost podizanja adekvatnog fasciokutanog, distalno baziranog režnja, čime se omogućava rekonstrukcija mekotkivnih defekata distalne trećine potkolenice, maleolarnih regija i dorzuma stopala.

Ključne reči:
noga; meka tkiva, povrede; lečenje; hirurgija, rekonstruktivna, procedure; režnjevi, hirurški; anatomija, topografska.

Correspondence to: Milomir Gačević, Military Medical Academy, Clinic for Plastic Surgery and Burns, Crnotravska 17, 11 040 Belgrade, Serbia. Phone: +381 11 3608 750, 381 63 342 660. E-mail: adonisscg@excite.com

Introduction

Blast injuries to the lower extremities and the feet inflicted with a large number of landmines and explosive ordnance used in modern warfare, as well as injuries resulting from industrial, traffic or similar accidents are frequently seen today.

The complexity of the management of lower leg and foot injuries accompanied by soft tissue defects arises from the anatomical specificity of the region, localization area, the size and structure of tissue defect.

The latest knowledge of anatomy, concerning particularly the structure of vascularization of some compartments, makes this area, in terms of reconstruction, very specific.

The anterior surface of the tibia is covered with a thin layer of the skin and subcutaneous tissue, so, defects to this part are ussually accopmanied by the exposed bone without periosteum, due to what an autotransplant is excluded as a method of surgical treatment [1]. Poor elasticity of the lower leg outer layer and foot skin limits its ability for transfer.

Tendons, neurovascular structures and bones are seated immediately under the skin in the distal third of the lower leg. However, reconstructive surgery requires application of full- thickness skin grafts which could hardly be harvested from that region [2–5].

The most distal part of the lower leg, the malleolar region as well as the Achilles tendon area still present a serious reconstructive problem [4].

So far, some attempts have been made to bridge the defects in the malleolar and Achilles tendon area with grafts obtained from the foot *dorsum* [2, 6–9], and the middle third of the lower leg [4, 5, 10, 11]. All reverse fasciocutaneous flaps raised in the projection of magistral blood vessels (*arteria peroneae*, *a. tibialis posterior*, *a. tibialis anterior*, *a. dorsalis pedis*) have been used, but due to rotation of almost 180° they do not give satisfactory results, and the lack is that a large blood vessel is sacrified.

Numerous anatomical studies reporting great variations among the obtained results have been conducted to localize peroneal artery perforators more precisely [10, 12, 13]. Application of computed tomography (CT) angiography has been described in some recently published papers as well [14].

The aim of this study was to define vascularization fields of the skin and fascia vascularized by the ascending branch of the peroneal artery *ramus perforans* (PARP) in order to assess the level of reliability of the distal flap on the anterolateral aspect of the distal third lower leg.

Methods

This study involved 20 lower extemities from cadavers of both sex and various age. Age, sexual maturity, fully completed growing process, undiagnosed diabetes or some other vasculopathy in the lower extremity prior to death were the criteria we followed in selecting cadavers.

We applied two investigation methods, presuming that they would be sufficient to ensure reliable findings required for the assessment of the quality, design and size of a potential distally placed graft. The first method was injection of gentian violet dye with subsequent dissection of dyed tissue as potential graft material. The second method was corrosion preparation.

Prior to the application of either method, we recorded all sex- and age-related data, measured body height in centimeters (cm), and height (length) of the fibula bone in millimeters (mm).

Injection of gentian violet dye with subsequent dissection of dyed tissue as potential graft material

The selected cadavers (group 1) were positioned laterally on the opposite side to dissection. Proceeding from the principals of the topographic anatomy, we dissected 10 lower legs. Using lateral approach, we separated the peroneal artery 15 cm in length measured from the distal point of the lateral malleolus proximally, clearly localized the point of bifurcation of the perforating branch, and tied off the peroneal artery 2 cm below the bifurcation. A transverse arteriotomy was then made on the peroneal artery 5–10 cm above the bifurcation of *ramus perforans*, and a braunila (Romed 20G) was placed (Figure 1a). Firstly, we injected 10 cm^3 of lukewarm water (45ºC), and then 20 cm^3 of solution containing 95% gentian violet and 5% gelatine at temperature of 40ºC (Figure 1, b and c). An antilaterally marked surface of the skin on the distal third of the lower leg was, thus, obtained, and was clearly inked (red or black ink).

We then laid a transparent foil directly on the limited area of the skin and took an actual-size photo of it (Figure 1d). Upon reviewing the skin areas marked by gentian violet on the same legs, we started with separation and raising of the skin and fascia as ascendent branch of the PARP–based fasciocutaneous flap. Separation was done under magnification with Keller eyeglasses.

The marked surface area on the anterolateral side of the foot was not measured, it was the vascularization field of the distal branch of the PARP.

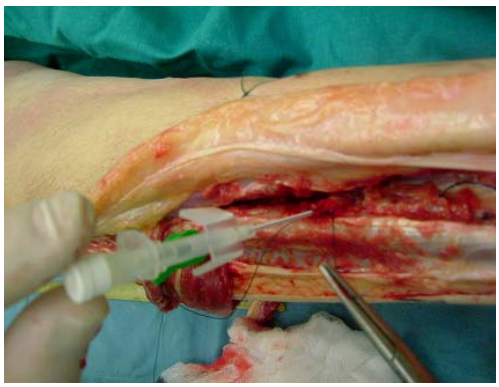
We measured the medial height of the origin of perforating branch from the inferior border of the lateral malleolus, the medium length of the marked area, the medium width and medium area of the stained segment (Figure 1, e–g).

The area of the skin labeled by gentian violet dye was measured in mm^2 using CAMIA software (Figure 2).

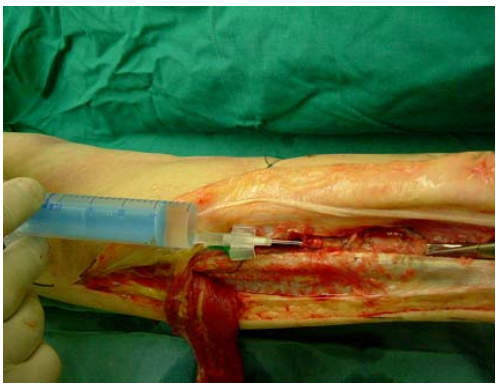
Corrosion preparation

We made corrosion preparations in the 10 lower legs of cadavers from the group 2 at the Anatomic Institute, School of Medicine Belgrade in the following way:

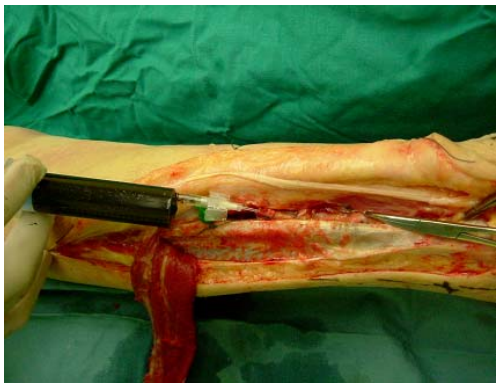
We separated popliteal artery at the level of popliteal fossa before it bifurcates into its terminal branches and firstly injected 50 cm^3 of lukewarm water to rinse even the tiniest blood vessels, and then 50–70 cm^3 of methyl metacrylate. When it became consolidated, after three weeks, we obtained a corrosive preparation by maceration, i.e. the prints of blood vessels of the lower leg and foot (Figure 3).



a – placement of braunila into the peroneal artery



b – insertion of lukewarm water into the peroneal artery



c – insertion of a gentian violet dye into the peroneal artery

d – marked surface area of a potential flap upon insertion of a gentian violet dye on the anterolateral side of the lower leg

e – measurement of the flap length bifurcation

f – the height of the perforating branch measured from the lateral malleolus

Fig. 1 – Injection of gentian violet dye with subsequent dissection of the stained tissue as potential graft material

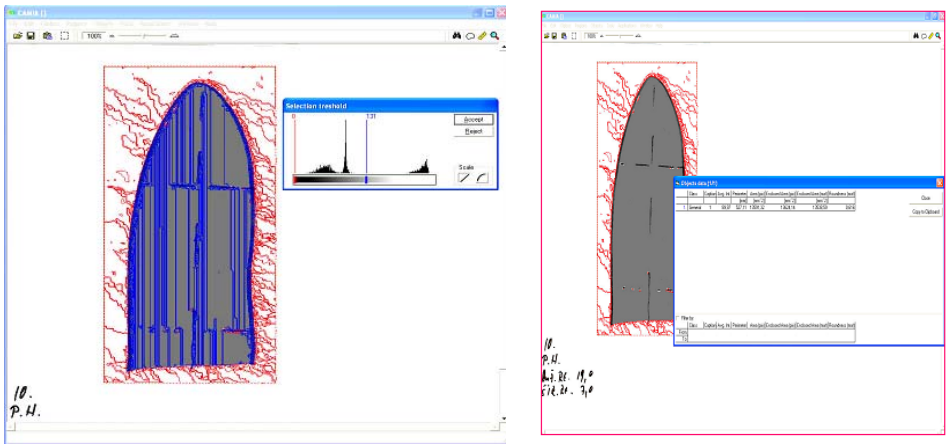
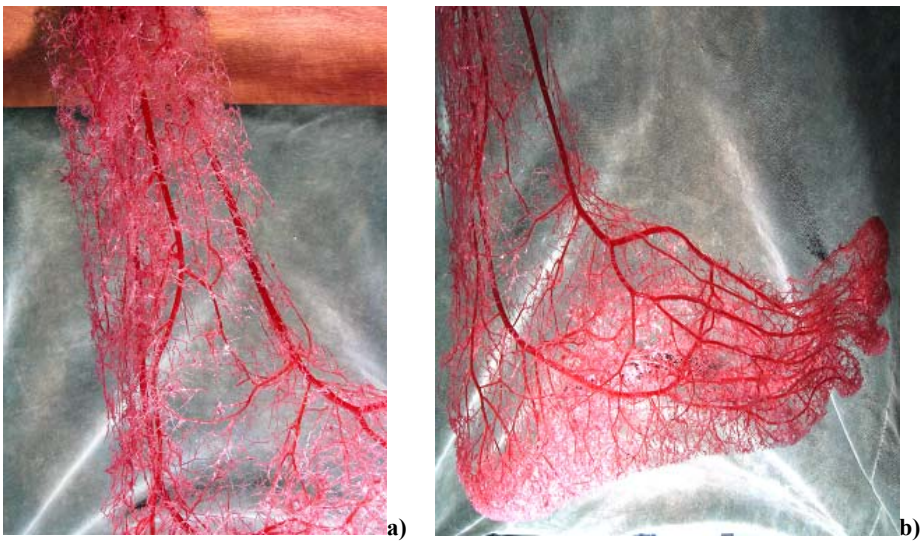


Fig. 2 – Computer-based calculations of the flap surface area



a) b)

Fig. 3 – Corrosion preparation for the lower leg (a) and for the foot (b)

This technique enabled us to obtain even more precise anastomoses of vessels and the adjacent vascular territories as well as the internal diameters of the viewed blood vessels.

Results

The method of injection of gentian violet was applied on 10 lower legs harvested from 10 cadavers (9 males and 1 female). We always used one lower leg of one cadaver; the left leg in 6 cases and the right one in the other 4 cases.

Cadavers in the group 1 aged 22–69 years (the mean age 54 years), their body height ranged from 170–192 cm (the medium height 184.5 cm), and the length of the fibular bone averaged 467 mm (range, 410–500 mm) as presented in Table 1.

The measured parameters included the height of the origin of perforating branch from the inferior border of the lateral malleolus, and the length, width and area of the marked skin.

The length of the marked area of the skin from the origin of perforator, distally to the top proximally, was measured to range 125–210 mm, with an average of 164 mm, while its width averaged 66 mm (range 57–77 mm). At the same time, using a special computer technique, the marked skin area was calculated to range 6 385 mm^2–14 341 mm^2, with an average of 10 305 mm^2. The mean height of the *ramus perforans* measured origin from the inferior border of the lateral malleolus was 66 mm (range 39–82 mm) (Table 1).

Age, sex, body height and length of fibula of the studied cadavers from the group 2 are presented in Table 2.

Table 1

Characteristic parameters of cadavers in the group 1 (the method with gentian violet dye)

Parameter	n	$\bar{x}$ ± SD (range)
Sex		
male/ female	9/ 1	
Age (years)		54 ± 13 (22–69)
Body height (cm)		185 ± 7 (170–192)
Fibula length (mm)		468 ± 27 (410–500)
The perforator origin height from malleolus (mm)		66 ± 13 (39–82)
Flap length (mm)		164 ± 26 (125–210)
Flap width (mm)		66 ± 6 (57–77)
Flap surface area (mm^2)		10,305 ± 2,763 (6,385–14,341)

Table 2

Characteristic parameters of cadavers in the group 2 (the corrosion preparation)

Parameter	n	$\bar{x}$ ± SD (range)
Sex		
male/ female	7/ 3	
Age (years)		50 ± 13 (27–68)
Body height (cm)		180 ± 8 (174–192)
Fibula length (mm)		417 ± 27 (335–445)
Diameter of the PARP* before bufurcation (mm)		1.37 ± 0.21 (1–1.8)
Diameter of the ascending branch after bifurcation (mm)		1 ± 0.17 (0.8–1.2)
Diameter of the descending branch after bifurcation (mm)		0.99 ± 0.19 (50.7–1.3)
Length of the ascending branch after bifurcation (mm)		139 ± 24 (100–180)
Communication with the *arteria tibialis anterior* (mm)		102 ± 16 (75–128)

PARP – peroneal artery *ramus perforans

By applying this technique, we obtained very precise results and presented them in Table 2 as follows: length of the perforating branch to the point where it bifurcates into the ascending and descending branches; the diameter of the perforating branch before its bifurcation; height of the communication between the ascending branch and *a. tibialis anterior*; diameter of the ascending branch after its bifurcation; diameter of the descending branch after its bifurcation; communication between the ascending and descending branch and the adjacent blood vessels.

The parameters obtained by this method were as follows: the diameter of the perforating branch before its bifurcation was calculated to be 1.37 mm (1.0–1.8 mm); the diameter of the ascending branch after its bifurcation averaged 1.0 mm (0.8–1.2 mm) and the diameter of the descending branch was 0.99 mm (0.7–1.3 mm). The mean length of the ascending branch was 139 mm (range 120–180 mm) and the height of its communication with the ATA ranged from 75 to128 mm, with an average of 102 mm (Table 2).

Figure 4 shows the view of distally-based flap on the ascending branch of the PARP.

Discussion

The lower leg reconstruction presents a great challenge to the surgeons due to the simple reason that the amount of soft tissue available for reconstruction is insufficient.

The insufficiency in tissue is not reflected in the lack of the available skin cover only but in poorly developed subcutaneous tissue and a small number of muscles for reconstruction of some defect [15].

It is important to emphasize that during the war waged on the territory of the former Yugoslavia the wounds to the lower legs and foot (mostly in Bosnia and Herzegovina) were the most common due to the specific characteristics of the warfare and the use of a large number of explosive ordnances and contact mines. These wounds are most often seen in industry, and situations such as car accidents.

The Pontén's [16] research (1981) and his findings that the subcutaneous, deep fascia is very rich in blood vessels of the perforators of the anterior and posterior tibial artery, and that *a. peronea* provides raising of reliable fasciocutaneous flaps with the length to width of the base ratio of 2.5–3:1 [16].

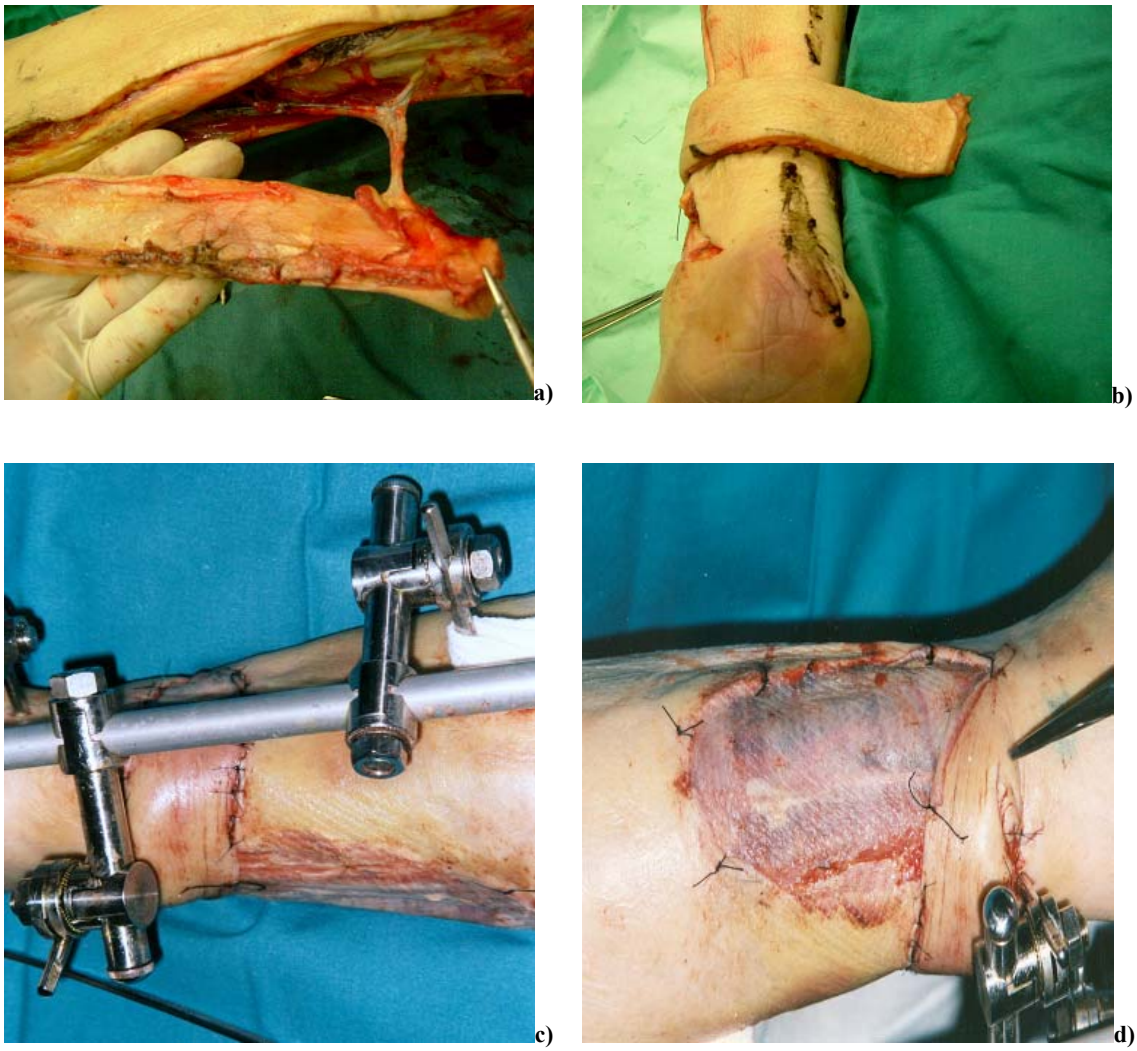


Fig. 4 – The view of a distally-based flap on the ascending branch of the peroneal artery *ramus perforans* (PARP): a – the bifurcation point of the ascending and discending branch of the PARP; b – flap reconstruction possibility; c and d – clinical appliction of the flap (the secondary defect reconstructed with the autotransplant)

At the end of the 1980s, the first papers describing distally-based, reverse and recurrent flaps appeared in the literature, that to a great extent facilitated a high-quality management of soft-tissue defects of the lower arm and foot [17–20].

These distally-based flaps are fasciocutaneous, that makes them reliable, with the base to length ratio of even 1 : 3–4. Contrary to the reverse-flow flaps, recurrent flaps have the physiological entry into the circulation, that secures distal position and makes them highly reliable.

One of those distally-based flaps is a flap based upon the ascending branch of the PARP. Circulation in this flap is recurrent, meaning that it has a physiological flow and a base on the anterolateral aspect of the lower arm, that it is vascularized *via* the recurrent, ascending branch of the PARP [12]. That recurrent circulation was in fact the topic of our investigation.

Published and available papers clearly show the presence of the ascending branch, but not its basic parameters such as diameter, length and probable communication with the adjacent blood vessels. The first paper to mention diameters of this distally-placed flap was published by Chang et al. [12] in 2004. In the series of 7 patients, they elevated the flaps of 17 × 6 cm in size on average and called them the new fasciocutaneous distally-placed flaps. They introduced them into the clinical practice.

Well aware that reconstruction procedures involving the region of the distal third of the lower leg are very limited [13, 21, 22], our aim was to clearly define a potential flap and its application.

The cadaver age-related data proved to be very remarkable, since the vascularization of a potential flap is directly proprtional to the age. In other words, the older a cadaver, the smaller the length, width, and the surface of the marked skin. It is also known that the elasticity of blood vessels decreases, and their diameter is smaller in proportion to age.

The height of a cadaver was found to be another significant data indicating size of a potential flap. During the investigation, our results revealed a direct proportion between the height and size of a flap. It is also important to emphasize that the point from which the *ramus perforans* branches off the peroneal artery, measured from the lower edge of the lateral malleolus was also proprotional to the height of a patient. The important data concerning the cadaver's height is the point at which the ascending branch communicates with *a. tibialis anterior*. The measured values showed that the height of communication was directly proportional to the height of a cadaver.

Observing these parameters (age, height, tibular bone length) we found them to be very crucial for potential use of a flap in the clinical practice. It is clear that we should consider the patient's age, but in designing a flap, it is important to know at what level *ramus perforans* bifurcates, because those parameters indicate the size of a flap we could harvest, and the diameter of a defect and its localization that we can reconstruct.

Blood vessels in the group A, instilled with the mixture of gentian and gelatine, enabled us to adequatelly measure length and diameter of the perforator and its two branches. At the same time, marking of the skin on the lateroanterial aspect of the lower leg showed the maximum dimensions, including both length and width, of a potential flap.

It should also be pointed out that, during instillation of gentian violete, we observed the changes in color in the distal two-thirds of a potential flap after instillation of the first 20 cm^3. The color change in the upper third occured after instillation of the second 20 cm^3 under a higher pressure. We assumed that it was that height or length of the ascending blood vessel where it communicated with *a. tibialis anterior*, and that the proximal third was a part lying over that communication. In this case as well, the height of bifurcation of the perforating branch was proportional to the cadaver's height and the length of fibula.

The average length of a potential flap in those 10 lower legs, measured from the height of the bifurcation of the perforating branch proximally, was 164 mm (125–210 mm). Our expectation to obtain longer marked skin areas from higher cadavers and those with a longer fibula was fully confirmed.

If we compare the obtained data, it is clear that this flap by its length, width and design could be completely used for reconstruction of lateral malleolus defects, since the rotation point (pivot) was only 6.6 mm, proximal to the lower edge, and the average length of the marked surface was considerably larger. The distance of a potential flap rotation point from the medial malleolus was less than 7 cm, that makes it more convinient for reconstruction of medial malleolus defects as well.

The average width of potential flaps in the same 10 lower legs was 66 mm (57–77 mm). However, it should be emphasized that the length was clearly limited, whilst the width was asymmetrical. The widest part of a potential flap was 7–10 cm from the lower distal measurement point. In other words, the largest flap width was found at the level of communication between the ascending artery and *a. tibialis anterior*.

Upon completion of outer measurements of those 10 lower legs, we started dissection and elevation of the marked skin and fascia as fasciocutaneous flaps. During dissection, we closely followed the course and the length of the ascending branch of the perforator as the basic vascular branch of the flap, as well as the diameter of the ascending and discending branches at their origin immediately after their point of bifurcation.

The ascending branch is situated on the inner side of the deep fascia; on its recurrent course, it gives off a large number of small blood vessels which partly penetrate the fascia and pass into the subcutaneous region pushing their terminal branches towards the skin surface. Running from distal to proximal, this blood vessel is lost in the network of tiny blood vessels and capillaries. That visual effect completely matches the area of the marked skin.

By its characteristics, the obtained fasciocutaneous flap covered a great part of the distal third of the lower leg, including a part of the area of the Achilles tendon insertion as we have shown in this study.

The obtained parameters were compared with the available data in the literature.

The height of the perforator bifurcation point measured, as well, from the distal plane of the lateral malleous in cadavers (20 cadaveric lower legs) by Beveridge et al. [10] was 44–90 mm in diameter (the average of 67 mm). In the group of 10 cadavers, we measured the same height which averaged 66 mm.

Based on the data obtained by the same authors, the length of the perforator from its origin down through the interosseous membrane and up to the bifurcation point was 65 mm. We obtained the average length of 51.7 mm during our investigation.

The mentioned authors measured the diameter of ascending and discending branches shortly after bifurcation from the same tree in the same group of cadavers. The average diameter of ascending and descending branches were 10 mm and 11 mm, respectively. They came to the conclusion that the descending branch appeared to be stronger, and that it communicated with the terminal branches of the peroneal artery (*aa. maleolares*) and lateral branches (*a. dorsalis pedis*) forming, thus, a strong lateral malleolar *plexus*. At the same time, they pointed out that it is possible to design a distally-based flap that can be very reliable. It might be concluded from the presented that the obtained results are very compatible, with an insignificant discrepancy in measurements of the diameter of those two branches.

Our investigation proved that it might be possible to elevate a reliable, distally-based flap from the base in the direction of lateral malleolus. Our investigation confirmed that in the early 2010, Li et al. [23] used this flap for reconstruction of knee defects with microvascular anastomosis.

The method of making preparations is a very precise and reliable procedure which provided us with all the data significant for assessing the possibility of raising a distally-based reverse-flow flap vascularized by the ascending branch of the PARP and its use in the clinical practice.

This method was thoroughly applied in 10 lower legs at the Anatomic Institute of the School of Medicine. The main point of the method is to obtain the smallest blood vessel print of the lower leg and foot which relate to the actual anatomy, position and diameter.

The obtained preparation enabled us to precisely measure all the required parameters for assessing the quality of the analyzed blood vessel and its position and relationship to the adjacent blood vessels.

The diameter of the perforating branch before its bifurcations into ascending and descending branches averaged 1.37 mm (1.0–1.8 mm) in those 10 lower legs.

The diameter of the ascending branch at the level of its bifurcation from the main tree was 1.00 mm, whilst the diameter of the descending branch at its origin measured 0.99 mm. By this measurement, we found the diameter of the ascending branch to be minimally increased for 0.01 mm only.

Latest investigations conducted by Ribuffo et al. [14] using CT and a very sensitive Doppler sonography showed that the average diameter of *ramus perforans* was 1.91 mm, and the diameter of the first part of the ascending branch was 0.8 mm, that almost corresponded to the measurement results we obtained during the investigation.

The height at which the ascending branch communicates with the anterior tibial artery was 75–128 mm (the average of 102 mm).

In the precise study of the corrosive preparation, we observed that the terminal branches including both ascending and descending ones, give off a large number of tiny blood vessels to supply deep fascia and the overlying skin. The next issue observed was that they both communicate with *a. tibialis anterior*. The descending branch participates in the formation of the lateral malleolar plexus and communicates as well with the terminal branches of peroneal artery through *rami lateralis*.

Beveridge et al. [10] also very accurately described the communication of the descending branch of *ramus perforans* and its participation in the formation of the *rete maleolaris lateralis* as well as the possibilities of reverse-flow vascularization.

Based on those investigations, statistical data processing and the comparison of some methods, we determined that *ramus perforans* of the peroneal artery is a constant branch which branched off the peroneal artery at the average height of 57 mm, measured from the lower edge of the lateral malleous, pierced the interosseous membrane, and after transition of the average 47 mm and the diameter of 1.37 mm on transition, *ramus perforans* divided into an ascending and a descending branch, and that the ascending branch communicated with the anterior tibial artery in 90% of cases. By precise measuring and comparing the ratio between the length from the lateral malleolus to the rotation point (pivot point) of that flap, and from the rotation point to its tip, we could conclude that a flap is convenient for covering a defect exactly at the lateral malleolus level. By simultaneous measuring the length from the medial malleolus and Achilles tendon at the same level, we obtained the same results which indicated the possibility of using this flap for covering defects in those regions.

With respect to the clinical application of this flap, Xia et al. [24] in their study in 2009, reported the edge necrosis as a complication that occured in 2 of 22 patients. Statistical processing of the measured data revealed that the height at which *ramus perforans* branches off the peroneal artery, measured from the distal plane of the lateral malleolus, was 1/8 of the total length of the fibula. This data was found to be very essential for clinical planning and design of a potential flap based on *ramus perforans*, what was clinically confirmed by our study results (Figures 4, c and d).

We also confirmed that the length of the ascending branch from the bifurcation point to the point of communication with the tibial artery was 1/5 of the total length of the fibula, that confirms the possibility of raising a reliable distally-based flap.

Conclusion

Based on the obtained findings analyses, the following could be concluded:

The peroneal artery is a constant blood vessel which supplies blood *via* its *ramus perforans* to a certain part of the

lateral and antero-lateral surface of the lower leg. R*amus perforans* branches off at the average height of 66 mm from the lower edge of the lateral malleolus, penetrates immediately the interosseous membrane, and bifurcates into two terminal branches: *ramus ascendens i ramus descendens.* Ascending branch provides nutrition and blood supply to a flap of an average length of 164 mm, and width of 66 mm.

By analyzing all the obtained data and parameters, we could conclude that the length of a potential flap is directly proportional to the height of a pateint, and what might be even more important, the height of *ramus perforans* bifurcation point is also proportional to the patient's height.

This proportionality was also found to the length of tibia as a local comparative parameter what can help us in the evaluation of the height of the perforator bifurcation, and the base of the distally based flap in its clinical application. The investigation of the vascularization of the ascending branch of the PARP led us to a conclusion that it is possible to raise a flap on the anterolateral side of the lower leg, distally based on this perforating branch of the peroneal artery, and that it can be used in clinical practice to treat soft tissue defects of the distal third of the lower leg, both maleolar regions, and the region of the insertion of the Achilles tendon and at the dorsal region of the foot.

Clinical application of this flap cannot endager any magistral blood vessel. It is easy to raise the flap with a good knowledge of its anatomy. The flap is easy to design, it is very mobile and reliable for skin reconstruction.

R E F E R E N C E S

1. *Panajtovic Lj, Kozarski J, Krtinic-Rapaic S, Stanojevic B.* Management of war burns of the lower leg using free flaps. Vojnosanit Pregl 2003; 60(6): 741–5.
2. *Valenti P, Masquelet AC, Romana C, Nordin JY.* Technical refinement of the lateral supramalleolar flap. Br J Plast Surg 1991; 44(6): 459–62.
3. *Koshima I, Itoh S, Nanba Y, Tsutsui T, Takahashi Y.* Medial and lateral malleolar perforator flaps for repair of defects around the ankle. Ann Plast Surg 2003; 51(6): 579–83.
4. *Koshima I, Nanba Y, Tsutsui T, Takahashi Y, Itoh S.* Perforator flaps in lower extremity reconstruction. Handchir Mikrochir Plast Chir 2002; 34(4): 251–6.
5. *Lee S, Estela CM, Burd A.* The lateral distally based adipofascial flap of the lower limb. Br J Plast Surg 2001; 54(4): 303–9.
6. *Ali MW, Mohajir AM.* Dorsalis pedis artery: variations and clinical significance. J Indian Med Assoc 1996; 94(11): 417–8.
7. *Converse JM.* Reconstructive plastic surgery. 2nd ed. Philadelphia: W.B. Sounders Company; 1977.
8. *Lawrence SJ, Botte MJ.* The deep peroneal nerve in the foot and ankle: an anatomic study. Foot Ankle Int 1995; 16(11): 724–8.
9. *Cormack GC, Lamberty BG.* A classification of fascio-cutaneous flaps according to their patterns of vascularisation. Br J Plast Surg 1984; 37(1): 80–7.
10. *Beveridge J, Masquelet AC, Romana MC, Vinh TS.* Anatomic basis of a fascio-cutaneous flap supplied by the perforating branch of the peroneal artery. Surg Radiol Anat 1988; 10(3): 195–9.
11. *Le Nen D, Beal D, Person H, Lefevre C, Sénécail B.* Anatomical basis of a fascio-cutaneous pedicle flap based on the inferolateral collateral artery of the leg. Surg Radiol Anat 1994; 16(1): 3–8.
12. *Chang SM, Zhang F, Yu GR, Hou CL, Gu YD.* Modified distally based peroneal artery perforator flap for reconstruction of foot and ankle. Microsurgery 2004; 24(6): 430–6.
13. *Kawamura K, Yajima H, Kobata Y, Shigematsu K, Takakura Y.* Clinical applications of free soleus and peroneal perforator flaps. Plast Reconstr Surg 2005; 115(1): 114–9.
14. *Ribuffo D, Atzeni M, Saba L, Guerra M, Mallarini G, Proto EB*, et al. Clinical study of peroneal artery perforators with computed tomographic angiography: implications for fibular flap harvest. Surg Radiol Anat 2010; 32(4): 329–34.
15. *Novaković M.* Vaskularized fibular graft. Belgrade: Zadužbina Andrejević; 2000. (Serbian)
16. *Pontén B.* The fasciocutaneous flap: its use in soft tissue defects of the lower leg. Br J Plast Surg 1981; 34(2): 215–20.
17. *Lamberty BGH.* Use of fasciocutaneous flaps in lower extreity reconstruction. Perspec Plast Surg 1990: 4: 146–51.
18. *Tolhurst DE.* Surgical indications for fasciocutaneous flaps. Ann Plast Surg 1984; 13(6): 495–503.
19. *Tolhurst DE, Haeseker B, Zeeman RJ.* The development of the fasciocutaneous flap and its clinical applications. Plast Reconstr Surg 1983; 71(5): 597–606.
20. *Amarante J, Costa H, Reis J, Soares R.* A new distally based fasciocutaneous flap of the leg. Br J Plast Surg 1986; 39(3): 338–40.
21. *Demirtas Y, Ayhan S, Sariguney Y, Findikcioglu F, Cukurluoglu O, Latifoglu O,* et al. Distally based lateral and medial leg adipofascial flaps: need for caution with old, diabetic patients. Plast Reconstr Surg 2006; 117(1): 272–6.
22. *Ahmed SK, Hashmi PM.* Delayed supramalleolar flap-an innovative technique for enhanced viability. J Ayub Med Coll Abbottabad 2005; 17(3): 76–9.
23. *Li F, Zeng B, Fan C, Chai Y, Ruan H, Cai P.* Distally based extended peroneal artery septocutaneous perforator cross-bridge flap without microvascular anastomoses for reconstruction of contralateral leg and foot soft tissue defects. J Reconstr Microsurg 2010; 26(4): 243–9.
24. *Xia ZB, Wang D, Yuan YJ, Min JK, Yang WL, Li HC,* et al. Repair of soft tissue defects on the lower limbs with the facial pedicled flap with vascular perforating branch of leg. Zhongguo Gu Shang 2009; 22(11): 853–5. (Chinese)

Received on June 16, 2010.
Revised on February 2, 2011.
Accepted on February 7, 2011.

ORIGINALNI ČLANAK

UDC: 618.1- 007.44-089
DOI: 10.2298/VSP1107583A

Upotreba transvaginalne mrežice u korekciji prolapsa pelvičnih organa kao minimalno invazivnog hirurškog postupka

Transvaginal mesh in repair of pelvic organs prolapse as a minimally invasive surgical procedure

Rajka Argirović, Milica Berisavac, Ivana Likić-Lađević, Saša Kadija, Vladimir Bošković, Vojislav Žižić

Klinički centar Srbije, Institut za ginekologiju i akušerstvo,
Beograd Srbija

Apstrakt

Uvod/Cilj. Prolaps genitalnih organa sa ili bez urinarne stres inkontinencije najčešći je zdravstveni problem starije ženske populacije i povećava se sa godinama starosti. Cilj rada bio je prikaz retrospektivne studije, koja analizira perioperativne i kasne postoperativne komplikacije kod upotrebe transvaginalne neresorptivne mrežice u rekontrukciji karličnog dna zbog prolapsa pelvičnih organa. **Metode.** Podaci su dobijeni retrospektivnom analizom 96 bolesnica koje su operisane u periodu septembar 2006 – januar 2010. Za korekciju spada korišćen je *Prolift* komplet proizvođača Gynecare, Ethicon, USA. **Rezultati.** Sve bolesnice imale su prolaps u stadijumu III ili IV. Prednji *prolift* ugrađen je kod 52 (54%) bolesnice, zadnji kod 32 (33%), a totalni kod 12 (13%) bolesnica. Imali smo samo jednu ozbiljnu intraoperativnu komplikaciju, povredu mokraćne bešike, koja je odmah prepoznata i sanirana. Drugih ozbiljnih intraoperativnih komplikacija nije bilo. Kasni neželjeni efekti, koji su se manifestovali tri meseca nakon operacije, bili su: erozija vagine kod devet (9,3%) bolesnica, retrakcija mrežice kod šest (6,2%) bolesnica, *de novo* inkontinencija kod pet (5,2%) bolesnica. Sve bolesnice bile su dostupne praćenju. Prosečno vreme praćenja iznosilo je 18,2 meseca (3–35). Uspešan ishod tretmana postignut je kod 93,75% bolesnica, a kod šest bolesnica (6,25%) pojavili su se recidivi. **Zaključak.** Upotreba neresorptivne polipropilenske mrežice predstavlja minimalno invazivnu i prilično sigurnu metodu za rekontrukciju karličnog dna. Iako ukupan morbiditet nije mali, napominjemo da je 69% bolesnica u studiji operisano zbog recidiva prethodne klasične operacije. Pored toga, ovo su prvi rezultati nove operativne tehnike bez prethodnog iskustva.

Ključne reči:
karlični organi, prolaps; hirurška mrežica; vagina; hirurgija, ginekološka, procedure; lečenje, ishod.

Abstract

Background/Aim. Prolapse of genital organs with or without urinary stress incontinention is the most often health problem in the elderly female population tending to increase with ageing. The aim of this study was to assess the perioperative complications and short-term outcomes of prolaps repair using transvaginal polypropylene mesh (Prolift system, Gynecare, Ethicon, USA). **Methods.** A retrospective study was conducted evaluating 96 women from September 2006 to January 2010 who undewent vaginal repair with implatation of a soft mesh manufactured by Gynecare, Ethicon, USA. **Results.** All the patients had a stage 3 or stage 4 prolapse according to the POP-Q system of ICS. Total mesh was used in 12 (13%) patients isolated anterior mesh in 52 (54%) patients and isolated posterior mesh in 32 (33%) patients. We reported one intra-operative bladder injury and no other serious complications. At 3 months, all 96 patients were available for follow-up. Vaginal erosion occured in 9 (9.3%) patients, shrinkage of mesh in 6 (6.2%) patients and *de novo* urinary incontinence in 5 (5.2%) patients. Failure rate was 6.25% (recurrent prolapse stage 3 or 4 even asymptomatic). **Conclusion.** Our study suggests that transvaginal polypropylene mesh applied with a tension-free technique is a safe and effective method with low intraoperative complications and low morbidity rates. However, some complications are serious and require highly specialised management.

Key words:
pelvic organ prolapse; surgical mesh; vagina; gynecologic surgical procedures; treatment outcome.

Correspondence to: Rajka Argirović, Institut za ginekologiju i akušerstvo KCS, Koste Todorovića 26, 11 000 Beograd, Srbija. Tel.: +381 63 27 82 74. E-mail: cvijiceva@yubc.net

Uvod

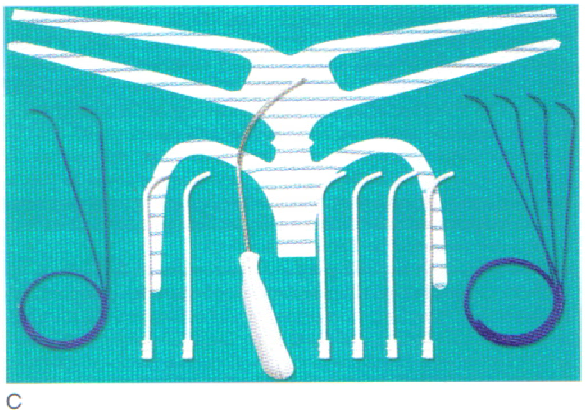
Prolaps genitalnih organa sa ili bez urinarne stres inkontinencije najčešći je zdravstveni problem starije ženske populacije i povećava se sa godinama starosti. Rizik od hirurške intervencije zbog prolapsa pelvičnih organa u opštoj ženskoj populaciji iznosi 11%, a 29% operisanih žena podvrgavaju se reoperaciji zbog neuspeha prve operacije tokom pet godina [1]. Klasične metode prednje i zadnje kolporafije ne daju trajne rezultate i dovode do velikog procenta relapsa. To je i bio razlog da se počne sa upotrebom različitih materijala u rekonstruktivnoj hirurgiji karličnog dna. U randomiziranoj studiji Webera i sar. [2] samo 30% bolesnica imalo je zadovoljavajuće rezultate sa tehnikom standardne kolporafije, dok je kombinovanje prednje kolporafije sa poliglaktinskom mrežicom dalo zadovoljavajuće rezultate kod 42% bolesnica. Pored biomaterijala, danas su najviše u upotrebi sintetske monofilamentne, polipropilenske mrežice sa makroporama, tako da se vrlo dobro integrišu u tkivo bez razlaganja. Polipropilenska mrežica prvo je počela da se koristi u ginekologiji za abdominalnu sakrokolpopeksiju, a potom za suburetralni sling na nivou srednje uretre, poznat kao beztenziona vaginalna traka (TVT). Koristeći tehniku sličnu transopturatornom slingu, Debodinance i sar. [3] 2004. godine upotrebili su polipropilensku mrežicu provlačeći krakove mrežice pomoću specijalne igle kroz opturatorni otvor za korekciju cistocele, a kada je reč o zadnjem segmentu male karlice, koristili su pararektalni prostor i provlačili krakove mrežice kroz oba sakrospinalna ligamenta. Poslednjih nekoliko godina objavljeni su brojni radovi sa velikim serijama bolesnika kod kojih su korišćene sintetske mrežice u vaginalnoj hirurgiji sa velikim procentom zadovoljavajućih rezultata (od 75 do 100%) i dužim srednjim praćenjem (od 1 do 36 meseci) [4–6].

Na našem tržištu trenutno imamo na raspolaganju monofilamentnu mrežicu proizvođača Ethicon Women's Health and Urology (Gynaecare Prolift System), koja je tako urađena da može da se koristi za rekonstrukciju prednjeg i/ili zadnjeg segmenata karličnog dna.

Cilj ovoga rada je da se opiše hirurška tehnika sa transvaginalnom beztenzionom mrežicom, da se saopšte intraoperativne i postoperativne komplikacije, kao i stepen uspešnosti novog hirurškog postupka.

Metode

Istraživanje je retrospektivno i obuhvata bolesnice operisane u Institutu za ginekologiju i akušerstvo Kliničkog centra Srbije u Beogradu u periodu od septembra 2006. do januara 2010. godine. U radu je prikazano 96 bolesnica kod kojih je u cilju rekonstrukcije karličnog dna upotrebljena neresorptivna meka mrežica od polipropilena (*Prolift kit*) proizvođača Gynecare, Ethicon, USA (slika 1). Bolesnicama je ugrađivan prednji, zadnji ili totalni *Prolift*, zavisno od stepena i tipa prolapsa. Korišćenje *Prolift* kompleta, uglavnom, bilo je namenjeno bolesnicama sa recidivima prethodnih rekonstruktivnih vaginalnih operacija ili onim bolesnicama sa velikim stadijumom primarnog prolapsa.



Sl. 1 – *Prolift* komplet

Preoperativna priprema obuhvatala je detaljnu anamnezu i popunjavanje upitnika koji je služio za grubu orijentaciju o tipu inkontinencije ukoliko je ona bila prisutna. Korišćen je upitnik *Bristol Lower Urinary Tract Symptoms* (BELUTS) koji obuhvata inkontinenciju, njen uticaj na seksualni život i na ukupan kvalitet života žene. Pored toga, obavezno je uzimana urinokultura i bakteriološki vaginalni bris, a bolesnice su se podvrgavale hirurškoj intervenciji tek po postizanju sterilnih nalaza. Ginekološki pregled obavljan je u dorzalnom litotomnom položaju, a stepen prolapsa procenjivan je pri kašlju ili izvođenju Valsalva manevra. Stepen prolapsa genitalnih organa stadiran je prema *pelvic organ prolapse – quantification* (POP-Q) sistemu [7]. Indikaciju za hiruršku korekciju predstavljao je prolaps vaginalnih zidova i/ili uterusa u stadijumu III i IV.

Pod perioperativnim komplikacijama podrazumevali smo komplikacije koje su se dešavale tokom hirurške intervencije i tokom prve četiri nedelje od operacije. Ukoliko je bilo potrebno, ugradnji mrežice pridruživani su odgovarajući hirurški postupci kao što je vaginalna histerektomija, prednja i/ili zadnja kolporafija ili *tension free vaginal tape obturator* (TVT-opturator). Kasne komplikacije bile su one koje smo registrovali nakon mesec dana od operacije ili kasnije.

Bolesnice su kontrolisane četiri nedelje posle operacije, a potom na tri i šest meseci i dalje svakih šest meseci. Kontrole su podrazumevale uzimanje podataka o subjektivnim tegobama i ginekološki pregled u dorzalnom litotomnom položaju sa testom kašljem ili Valsalva manevrom da bi se procenila uspešnost hirurškog postupka, odnosno stepen spada vaginalnih zidova i apeksa vagine shodno POP-Q sistemu. Minimalno vreme praćenja iznosilo je tri meseca. Pod recidivom smatran je prolaps u bilo kom segmentu u stadijumu III ili IV, čak i ako bolesnica nije imala nikakve simptome.

Mrežica koja je korišćena pri hirurškim postupcima je meka, monofilamentna, neresorptivna od prolena sa makroporama. Mrežica se sastoji od tri dela. Prednji deo se ostavlja između mokraćne bešike i vagine, a učvršćuje se sa po dva bočna kraka koja se provode kroz opturatorni foramen i arkus tendineus fascije pelvis (ATFP). Zadnji deo mrežice plasira se između rektuma i vagine i učvršćuje se sa po jednim krakom koji prolazi kroz ishiorektalnu jamu i *lig. sacrospinale*. Središnji deo podudara se sa vaginalnim apeksom i od-

vaja prednji od zadnjeg dela. Mrežici su pridodati instrumenti koji olakšavaju njeno ostavljanje na pravilan način. Kanula sa iglom koristi se da obezbedi optimalni prolazak mrežice kroz tkivo, sprečavajući povredu mišića i razaranje arkusa tendineusa fascije pelvis. Kroz kanulu provodi se vodič sa omčicom, koji obezbeđuje lako provlačenje krakova mrežice kroz prostor male karlice.

Bolesnica se postavlja u dorzalni litotomni položaj sa flektiranim nogama, tako da sa prednjim zidom abdomena čine ugao od oko 90º. U bešiku sa ostavlja Folijev kateter, a operativno polje se detaljno očisti antiseptičnim sredstvom.

Da bi se izbeglo veće krvarenje, savetuje se hidrodiskecija i zbog toga se obavezno infiltriše vaginalna sluzokoža fiziološkim rastvorom. U rastvor za hidrodisekciju ne dodaje se adrenalin.

Kod ostavljanja mrežice za prednji segment, načini se sagitalna kolpotomija koja polazi na 2 cm od grlića materice ili ožiljka na dnu vagine i završava se na oko 2 cm od spoljašnjeg meatusa uretre. Načini se lateralna disekcija mokraćne bešike, pri čemu se nastoji da se pubocervikalna fascija ostavi na zidu vagine. Smatra se da to obezbeđuje bolju podnošljivost mrežice i smanjuje procenat erozije vaginalne sluzokože. Otvaranjem paravezikalne fose identifikuje se ATFP, koji se pruža od zadnjeg zida gornjeg ramusa pubične kosti do spine išijadike. Načine se četiri incizije na koži vulve i to dve incizije na nivou anteromedijalnog ugla opturatornog otvora a u ravni spoljašnjeg otvora uretre i druge dve incizije 1 cm lateralno i 2 cm nadole u odnosu na prvu inciziju. Kroz pomenute incizije plasira se kanula pomoću igle, a kroz nju vodič sa omčicom, koji omogućava pravilno plasiranje mrežice. Pošto se mrežica pozicionira ispod bešike bez zatezanja, njen distalni kraj se fiksira za istmični deo uterusa sa nekoliko pojedinačnih šavova. Incizija na prednjem vaginalnom zidu ušije se produžnim šavom.

Za ostavljanje mrežice za zadnji segment, načini se zadnja kolpotomija. Odvoji se rektum od sluzokože zadnjeg vaginalnog zida, zadržavajući rektovaginalnu fasciju na sluzokoži vagine. Uđe se u pararektalni prostor obostrano i ispreparišu se oba sakrospinalna ligamenta. Na koži gluteusa načine se dve incizije 3 cm lateralno i 3 cm nadole od analnog otvora. Kanula sa iglom provodi se kroz ishiorektalnu jamu ispod nivoa levatora i izlazi kroz sam sakrospinalni ligament 2–3 cm medijalno od spine išijadike. Kada se postavi, mrežica za zadnji segment svojim centralnim delom nalazi se u rektovaginalnom prostoru, a proksimalni deo mrežice se fiksira za zadnji zid istmičnog dela uterusa. Potom se ukloni kanula i proveri zategnutost mrežice, a incizija na zadnjem vaginalnom zidu ušije se produžnim šavom. Po završetku obavezno je načiniti rektalni tuše da bi se utvrdio integritet zida rektuma ili postojanje ikakve strikture lumena rektuma od strane mrežice.

Sve bolesnice su dobijale profilaktički cefalosporine druge generacije 1 h pre operacije i terapija je nastavljana tokom 2 dana.

Rezultati

U analiziranom periodu *Gynecare Prolift* sistem upotrebili smo kod 96 bolesnica i to kod 52 za reparaciju prednjeg segmenta, kod 32 za zadnji segment, a kod 12 upotrebljen je totalni *Prolift*, odnosno prednji i zadnji. Prosečna starost bolesnica iznosila je 64 ± 7 godina. Preostale preoperativne karakteristike bolesnica prikazane su u tabeli 1.

Tabela 1

Karakteristike bolesnica

Karakteristike	Vrednosti
Godine starosti, $\bar{x}$ ± SD (raspon)	64 ± 7 (36–83)
Paritet, $\bar{x}$ ± SD (raspon)	1,8 (1–4)
Menopauzalni status, n (%)	84 (87,5)
Upotreba hormonske supstitucije, n (%)	8 (8,3)
Prethodna abdominalna histerektomija, n (%)	27 (28,1)
Prethodne vaginalne operacije, n (%)	39 (40,6)

Od ukupno 96 bolesnica, njih 56 (58,3%) imalo je ranije jednu operaciju u maloj karlici zbog istog problema, a 10 (10,4%) bolesnica imale su 2 ili više operacija zbog problema spada genitalnih organa. Kod preostalih 30 (31,2%) bolesnica upotreba sintetičkog grafta bila je prva operacija zbog problema poremećaja statike genitalnih organa. Kod 45 bolesnica nije prethodno urađena histerektomija. U toj grupi, uterus je konzervisan kod 37 bolesnica, a kod preostalih osam urađena je vaginalna histerektomija zbog benignih obolenja, najčešće mioma, ili na izričit zahtev bolesnice. U tabeli 2 prikazan je tip upotrebljene mrežice zavisno od inicijalnog prolapsa. Pridružene operacije prikazane su u tabeli 3. Kod 21 bolesnice učinjena je sakrospinalna fiksacija saglasno Nichols-ovoj tehnici [8] i to kod 10 bolesnica sa sačuvanim uterusom, a kod preostalih 11

Tabela 2

Mesto postavljanja mrežice saglasno sa inicijalnim prolapsom

Mesto postavljanja	*Prolift* A n = 52	*Prolift* P n = 32	*Prolift* A+P n = 12
Prednji segment			
st. III	20	0	3
st. IV	32	0	9
Srednji segment			
st. III	11	8	2
st. IV	14	21	10
Zadnji segment			
st. III	0	10	3
st. IV	0	22	9

A – *anterior* (prednji); P – *posterior* (zadnji); A + P – totalni

učinjena je fiksacija vaginalnog zida. U svim slučajevima rađena je jednostrana fiksacija za desni sakrospinalni ligament. Za ove postupke odlučivali smo se u slučajevima sa prednjim proliftom zbog cistocele velikog stepena, ali je bolesnica imala istovremeno i aplikalni defekt ili izraženu hipermobilnost uterusa. Suburetralni sling, zbog dokazane stres inkontinencije, učinjen je kod 26 (27%) bolesnica saglasno originalnoj tehnici de Leval-a od unutra ka spolja kroz opturatorni otvor [9].

Tabela 3

Pridružene operacije

Tip operacije	Bolesnice	
	n	%
Vaginalna histerektomija	8	8,3
Sakrospinalna fiksacija uterusa	10	10,4
Sakrospinalna fiksacija vagine	11	11,5
Obliteracija enerocele	13	13,5
TVT-O	26	27
Prednja kolporafija	6	6,2
Zadnja plastika	3	3,1
LAVH	1	1,04

TVT – O – *tension free vaginal tape obturator*
LAVH – *laparoscopically assisted vaginal histerectomy*

Prosečno trajanje operacije zavisilo je od tipa operacije, ali je za izolovan prednji ili zadnji *prolift* iznosilo 35 minuta, a za totalni *prolift* ili u situacijama sa pridruženim operacijama 90 min (65–170 min).

Najozbiljnija intraoperativna komplikacija bila je lezija mokraćne bešike kod jedne bolesnice, kod koje se radilo o trećoj operaciji na istom mestu. Povreda je prepoznata intraoperativno. Nije se odustalo od plasiranja mrežice, ali je bolesnica u postoperativnom periodu nešto duže nosila kateter. Nije bilo velikih intraoperativnih komplikacija kao što su povreda krvnih sudova, povreda rektuma, perifernih živaca ili obilnijeg krvarenja većeg od 500 mL.

Prosečno trajanje hospitalizacije iznosilo je 4,5 dana (2–14) dana. Sve bolesnice bile su dostupne za praćenje. U rad su bile uključene bolesnice sa minimalnim vremenom praćenja od tri meseca. Srednje vreme praćenja iznosilo je 18,2 meseca (3–35 meseci). Neposredne postoperativne komplikacije su se manifestovale kao infekcija urinarnog trakta kod četiri (4,2%) bolesnice, retencija urina kod 1 (1%) bolesnice i bol u preponi kod 2 (2,1%) bolesnice. Kasni neželjeni efekti koji su se pojavili tokom praćenja prikazani su u tabeli 4. Najčešći problem, koji se pojavljivao tokom prva tri meseca praćenja je ekspozicija mrežice u lumen vagine, odnosno erozija vagine kod devet (9,3%) bolesnica. Kod svih bolesnica erozija vagine bila je u prednjem segmentu i to u predelu gornje trećine vagine, kod jedne bolesnice nakon korekcije cistocele sa konzerviranim uterusom, a kod preostalih osam ekspozicija je nastala kod pridruženih vaginalnih histerektomija. Problem erozje vagine rešavan je kod sedam bolesnica u lokalnoj anesteziji, resekcijom dela mrežice koji prominira i resuturom vaginalne sluzokože. Kod dve bolesnice problem erozije rešen je lokalnom aplikacijom estrogenih krema. Nije bilo perzistentne erozije vagine.

Tabela 4

Postoperativni neželjeni efekti tri meseca nakon intervencije

Neželjeni efekti	Bolesnice	
	n	%
Erozija vagine	9	9,3
Retrakcija mrežice	6	6,2
De novo urinarna inkontinencija	5	5,2
Flatusna inkontinencija	1	1,04

Vrlo ozbiljnu komplikaciju koja se teško rešava predstavlja retrakcija mrežice, do koje je došlo kod šest (6,2%) bolesnica. Samo kod jedne nakon šest meseci bilo je neophodno delimično ukloniti mrežicu zbog izražene dispareunije. Ostalih pet žena bile su seksualno neaktivne i samo se pri pregledu pojavljivao bol i osećaj zategnutih kraka mrežice. *De novo* urinarna inkontinencija pojavila se kod 5 (5,2%) bolesnica i rešavana je suburetralnim slingom kroz opturatorni otvor. Jedna bolesnica imala je flatusnu inkontinenciju koja je iščežla spontano nakon šest meseci.

Pod neuspehom, odnosno recidivom, smatrali smo prolaps u stadijumu 3 ili 4 u bilo kom segmentu karličnog dna, bilo da je simptomatski ili asiptomatski. Procenat recidiva iznosio je 6,25% (6 bolesnica). Tri bolesnice imale su asimptomatski prolaps u prednjem segmentu, jedan nakon totalnog *prolifta* plasiranog u celini, a dva slučaja asimptomatske cistocele nakon zadnjeg *prolifta*. Takođe, tri bolesnice imale su recidiv u zadnjem segmentu, koji se manifestovao tri meseca nakon prednjeg *prolifta*, koji je samo kod jedne bolesnice bio simptomatski i zahtevao je naknadnu korekciju sa ugradnjom zadnjeg *prolifta*.

Diskusija

Tradicionalne tehnike za korekciju prolapsa, koje koriste postojeće tkivo i fascije, nažalost daju loše rezultate kroz duži vremenski period sa velikim procentom recidiva. Retrospektivna studija Olsena i sar. [1] pokazala je da taj procenat recidiva iznosi čak 29,2%.

Komplikacije posle upotrebe vaginalne poliprolpilenske mrežice u literaturi su loše opisane zbog nedostatka objektivne klasifikacije. Francuska TVM grupa [10] analizirajući svoje rezultate i komplikacije od 2000. godine, ukazuje na tri glavne grupe komplikacija: infekcija, retka ali ozbiljna komplikacija; vaginalna ekspozicija, najčešća komplikacija, uglavnom bez ozbiljnih posledica i retrakcija, teška komplikacija za rešavanje i malo opisana u literaturi.

Procenat recidiva u našoj studiji iznosio je 6,25% za srednji period praćenja od 18,2 meseca. Najčešće mesto recidiva bilo je u suprotnom segmentu od mesta ubacivanja mrežice. U literaturi postoje različita mišljena o kompletnoj rekonstrukciji karličnog dna ili reparacije samo na mestu oslabljene potpore [11] . Kod korekcije na mestu oslabljene potpore, postoji rizik od pojave *de novo* prolapsa i segmenta za koji se u momentu operacije smatralo da ima zadovoljavajući potporni mehanizam. Na osnovu rezultata prospektivne epidemiološke studije Clark-a i sar. [12] 60% slučajeva recidiva javlja se na istom anatomskom mestu, ukazujući direktno na

neuspeh primenjene hirurške tehnike, dok se 40% recidiva javlja u suprotnom segmentu, što ukazuje na promenu stabilnosti karličnog dna nakon hirurške intervencije.

Procenat recidiva u našoj studiji sličan je podacima iz literature. Fatton i sar. [4] našli su u seriji od 110 bolesnica recidiv od 4,7%, dok su Abdel-Fattah i Ramsay [13] u seriji od 289 bolesnica našli pojavu recidiva od 5%. Mesta recidiva u našoj studiji ukazuju na potrebu rekonstrukcije celog karličnog dna, s obzirom na to da su se recidivi uglavnom javljali u suprotnom segmentu od mesta insercije mrežice.

Prema podacima iz literature, procenat ekspozicije mrežice u vaginu kreće se od 5 do 30% [14, 15], ali ima radova koji pokazuju da uopšte ne dolazi do pojave ove komplikacije [16]. Najčešća komplikacija u našoj studiji bila je ekspozicija mrežice u vaginu, koja se javila kod devet bolesnica (9,3%) tokom prvih šest meseci praćenja. Mesto erozije vaginalne sluzokože bilo je u srednjoj liniji u gornjoj trećini vagine nakon korekcije cistocele. Kod osam bolesnica bila je istovremeno učinjena i vaginalna histerektomija. Vaginalna histerektomija predstavlja faktor rizika od pojave erozije vagine nakon ubacivanja mrežice. Prema podacima iz literature, ova komplikacija javlja se tri puta češće kada se učini istovremeno vaginalna histerektomija [17]. Postoji nekoliko faktora koji utiču na pojavu erozije vagine, a to su menopauzalni status, stepen prolapsa i, pre svega, iskustvo hirurga. Protektivni faktori za izbegavanje ove komplikacije su minimalna incizija vaginalne sluzokože, izbegavanje T–reza kod vaginalne histerektomije, brižljiva disekcija vaginalne sluzokože uz hidrodisekciju, izbegavanje čestih sutura i postavljanje mrežice ispod fascije [18].

De novo urinarna inkontinencija javila se kod pet (5,2%) bolesnica. Hiltunen i sar. [19] saopštili su učestalost *de novo* urinarne inkontinencije kod 14,3% bolesnica kod kojih je korišćena mrežica za korekciju cistocele, dok su Aungst i sar. [20] saopštili učestalost od čak 24,3%. Smatra se da na pojavu ove komplikacije utiče ekstenzivna disekcija vagine i paravezikalnog prostora, što verovatno doprinosi oštećenju uretralnog potpornog mehanizma i dovodi do denervacije i, konačno, insuficijencije unutrašnjeg sfinktera uretre.

Imali smo jednu bolesnicu sa flatusnom inkontinencijom nakon stavljanja mrežice za zadnji segment kod koje se problem spontano rešio nakon šest meseci. Inače, bolesnica je imala i druge probleme vezane za debelo crevo, a to je hronična opstipacija i divertikuloza, što je sve verovatno doprinelo pojavi ove komplikacije. Milani i sar. [21] objavili su pojavu *de novo* fekalne inkontinencije nakon insercije mrežice za zadnji segment.

Najozbiljnija komplikacija koju smo imali bila je retrakcija mrežice kod šest (6,2%) bolesnica, a samo kod jedne zahtevala je delimično uklanjanje mrežice šest meseci nakon operacije zbog prisutnih bolova i dispareunije. Nakon delimične resekcije mrežice bolovi su u potpunosti nestali. Retrakcija se javila kod dve bolesnice posle zadnjeg prolifta, a kod četiri nakon prednjeg prolifta. Retrakcija mrežice manifestuje se kao bol u vagini, koji se pojačava prilikom vaginalnog pregleda, dispareunija i fokalna osetljivost. Etiologija ove komplikacije nije u potpunosti poznata, ali se smatra da je posledica neadekvatne inkorporacije mrežice u okolno tkivo.

Postoje izvesni nedostaci u našoj studiji, a to je pre svega činjenica da nismo analizirali zadovoljstvo bolesnica, kvalitet života i seksualni život nakon insercije mrežice.

Da bi se utvrdio pravi efekat upotrebe mrežice u rekonstruktivnoj hrurgiji karličnog dna, potrebno je sprovesti randomiziranu studiju, u kojoj bi se uporedili rezultati tradicionalne hirurške tehnike i upotrebe sintetičkog grafta i pri tome analizirali rizik i dobre strane oba hirurška postupka.

Zaključak

Naši rezultati pokazuju da je upotreba mrežice, *prolift* kompleta, sigurna procedura za definitivnu korekciju prolapsa vaginalnih zidova. Pripada grupi minimalno invazivnih hirurških postupaka. U našoj studiji nismo imali ozbiljnih intraoperativnih i neposrednih postoperativnih komplikacija.

Upotreba sintetičkog grafta pred hirurga postavlja niz pitanja. Iako je sam hirurški postupak jednostavan, u postoperativnom periodu mogući su ozbiljni neželjeni efekti, od kojih se neki vrlo teško tretiraju. Iako ukupan postoperativni morbiditet nije mali, moramo istaći da je 69% bolesnica u našoj studiji operisano zbog recidiva prethodne operacije. Ožiljci i fibroza u predelu operativnog polja mogu negativno da utiču na ishod ponovne operacije. Pored toga, to su naši prvi rezultati upotrebe nove hirurške tehnike, bez prethodnog iskustva, što sigurno doprinosi većem postoperativnom morbiditetu.

L I T E R A T U R A

1. *Olsen AL, Smith VJ, Bergstrom JO, Colling JC, Clark AL.* Epidemiology of surgically managed pelvic organ prolapse and urinary incontinence. Obstet Gynecol 1997; 89(4): 501–6.
2. *Weber AM, Walters MD, Piedmonte MR, Ballard LA.* Anterior colporrhaphy: a randomized trial of three surgical techniques. Am J Obstet Gynecol 2001; 185(6): 1299–304.
3. *Debodinance P, Berrocal J, Clavé H, Cosson M, Garbin O, Jacquetin B,* et al. Changing attitudes on the surgical treatment of urogenital prolapse: birth of the tension-free vaginal mesh. J Gynecol Obstet Biol Reprod (Paris) 2004; 33(7): 577–88. (French)
4. *Fatton B, Amblard J, Debodinance P, Cosson M, Jacquetin B.* Transvaginal repair of genital prolapse: preliminary results of a new tension-free vaginal mesh (Prolift technique)-a case series multicentric study. Int Urogynecol J Pelvic Floor Dysfunct 2007; 18(7): 743–52.
5. *Gauruder-Burmester A, Koutouzidou P, Rohne J, Gronewold M, Tunn R.* Follow-up after polypropylene mesh repair of anterior and posterior compartments in patients with recurrent prolapse. Int Urogynecol J Pelvic Floor Dysfunct 2007; 18(9): 1059–64.
6. *Shah DK, Paul EM, Rastinehad AR, Eisenberg ER, Badlani GH.* Short-term outcome analysis of total pelvic reconstruction with mesh: the vaginal approach. J Urol 2004; 171(1): 261–3.
7. *Bump RC, Mattiasson A, Bø K, Brubaker LP, DeLancey JO, Klarskov P,* et al. The standardization of terminology of female pelvic organ prolapse and pelvic floor dysfunction. Am J Obstet Gynecol 1996; 175(1): 10–7.

8. *Nichols DH.* Sacrospinous fixation for massive eversion of the vagina. Am J Obstet Gynecol 1982; 142(7): 901–4.
9. *de Leval J.* Novel surgical technique for the treatment of female stress urinary incontinence: transobturator vaginal tape inside-out. Eur Urol 2003; 44(6): 724–30.
10. *Jacquetin B, Cosson M.* Complications of vaginal mesh: our experience. Int Urogynecol J Pelvic Floor Dysfunct 2009; 20(8): 893–6.
11. *Dwyer PL, O'Reilly BA.* Transvaginal repair of anterior and posterior compartment prolapse with Atrium polypropylene mesh. BJOG 2004; 111(8): 831–6.
12. *Clark AL, Gregory T, Smith VJ, Edwards R.* Epidemiologic evaluation of reoperation for surgically treated pelvic organ prolapse and urinary incontinence. Am J Obstet Gynecol 2003; 189(5): 1261–7.
13. *Abdel-Fattah M, Ramsay I.* Retrospective multicentre study of the new minimally invasive mesh repair devices for pelvic organ prolapse. BJOG 2008; 115(1): 22–30.
14. *Debodinance P, Cosson M, Burlet G, Engrand JB, Delporte P, Boulogne M,* et al. Tolerance of synthetic tissues and vaginal surgery. Report of 287 cases. J Gynecol Obstet Biol Reprod (Paris) 1999; 28(3): 216–24. (French)
15. *Collinet P, Belot F, Debodinance P, Ha Duc E, Lucot JP, Cosson M.* Transvaginal mesh technique for pelvic organ prolapse repair: mesh exposure management and risk factors. Int Urogynecol J Pelvic Floor Dysfunct 2006; 17(4): 315–20.
16. *Migliari R, De Angelis M, Madeddu G, Verdacchi T.* Tension-free vaginal mesh repair for anterior vaginal wall prolapse. Eur Urol 2000; 38(2): 151–5.
17. *de Tayrac R, Devoldere G, Renaudie J, Villard P, Guilbaud O, Eglin G .* Prolapse repair by vaginal route using a new protected low-weight polypropylene mesh: 1-year functional and anatomical outcome in a prospective multicentre study. Int Urogynecol J Pelvic Floor Dysfunct 2007; 18(3): 251–6.
18. *Cervigni M, Natale F, La Penna C, Panei M, Mako A.* Transvaginal cystocele repair with polypropylene mesh using a tension-free technique. Int Urogynecol J Pelvic Floor Dysfunct 2008; 19(4): 489–96.
19. *Hiltunen R, Nieminen K, Takala T, Heiskanen E, Merikari M, Niemi K,* et al. Low-weight polypropylene mesh for anterior vaginal wall prolapse: a randomized controlled trial. Obstet Gynecol 2007; 110(2 Pt 2): 455–62.
20. *Aungst MJ, Friedman EB, von Pechmann WS, Horbach NS, Welgoss JA.* De novo stress incontinence and pelvic muscle symptoms after transvaginal mesh repair. Am J Obstet Gynecol 2009l; 201(1): 73.e1–7.
21. *Milani R, Salvatore S, Soligo M, Pifarotti P, Meschia M, Cortese M.* Functional and anatomical outcome of anterior and posterior vaginal prolapse repair with prolene mesh. BJOG 2005; 112(1): 107–11.

Primljen 23. VI 2010.
Revidiran 20. VII 2010.
Prihvaćen 27. VII 2010

ORIGINAL ARTICLE

UDC: 618.11-006-079.4
DOI: 10.2298/VSP1107589T

Evaluation of the risk malignancy index diagnostic value in patients with adnexal masses

Procena dijagnostičke vrednosti indeksa rizika od malignosti kod bolesnica sa adneksalnim tumorima

Milan Terzić*†, Jelena Dotlić*, Ivana Likić Ladjević*, Jasmina Atanacković‡, Nebojša Ladjević†§

Clinical Center of Serbia, *Institute of Obstetrics and Gynecology, ‡Department of Pathology, §Center for anesthesiology and resuscitation, **Belgrade, Serbia; †University of Belgrade, School of Medicine, Belgrade, Serbia**

Abstract

Background/Aim. Ovarian cancer is the leading cause of death from gynecologic malignancies. Risk of malignancy index (RMI) is recommended in assessment of patients with adnexal masses. The aim of this study was to verify the effectiveness of the RMI in the discrimination between benign lesions and malignant adnexal masses in clinical practice. **Methods.** Ultrasounds were performed for all the patients and menopausal status, CA125 level and calculated RMI were defined. All the patients were divided into 3 groups depending on RMI (< 25, 25–200, > 200). After operations all adnexal masses were analyzed histopathologically (HP) and then sensitivity, specificity and predictive value of RMI were calculated. **Results.** Out of a total of 81 patients involved benign tumor had 51 (62.96%) and malignant 30 (37.04%) of the patients. The average value of CA125 in the group of patients with benign adnexal masses was 68.3 U/mL and in the group of patients with malignant adnexal masses it was 581.95 U/mL. In the group of patients with benign adnexal masses the average RMI was 284.9 and in the group of patients with malignant adnexal masses RMI was 469.2. All the results showed a positive correlation between both HP categories and RMI categories. The more malignant HP result produced higher RMI and the cut off value was RMI = 200. Sensitivity of RMI was 83.33%, specificity was 94.12%, positive predictive value was 89.29% and negative predictive value was 90.57%. **Conclusion.** Our study showed that RMI is very reliable in differentiation benign from malignant adnexal masses.

Key words:
ovarian neoplasms; diagnosis, differential; risk assessment; ultrasonography; menopause; histological techniques; postoperative period.

Apstrakt

Uvod/Cilj. Karcinom ovarijuma je vodeći uzrok smrti od ginekoloških maligniteta. U istraživanju bolesnica sa adneksalnim masama koristi se indeks rizika od malignosti (RMI). Cilj studije bio je ispitivanje efektivnosti RMI kao metode za razlikovanje benignih od malignih adneksalnih tumora u kliničkoj praksi. **Metode.** Svim bolesnicama urađen je ultrasonografski pregled organa male karlice, određen menopauzalni status i nivo tumorskog markera CA125, a zatim izračunat RMI. Bolesnice su bile podeljene u tri grupe prema vrednosti RMI (< 25, 25–200, > 200). Posle operativnog zahvata adneksalni tumori su histopatološki (HP) analizirani, a zatim su izračunate vrednosti senzitivnosti, specifičnosti i prediktivne vrednosti RMI. **Rezultati.** Od ukupno 81 ispitane bolesnice benigni tumor imala je 51 ispitanica (62,96%), dok je njih 30 (37,04%) imalo maligni tumor. Prosečna vrednost CA125 u grupi bolesnica sa benignim adneksalnim tumorima bila je 68,3 U/mL, dok su one sa malignim adneksalnim tumorima imale vrednost CA125 od 581,95 U/mL. Kod bolesnica sa benignim tumorima prosečan RMI bio je 284,9, a u grupi sa malignim tumorima 469,2. Rezultati pokazuju pozitivnu korelaciju između obe kategorije HP nalaza i kategorija RMI: što je veći RMI, HP nalaz pokazuje veću malignost, a značajna granična vrednost RMI je 200. Na osnovu rezultata ove studije, senzitivnost RMI iznosi 83,33%, specifičnost 94,12%, dok je pozitivna prediktivna vrednost 89,29%, a negativna prediktivna vrednost 90,57%. **Zaključak.** Naša studija pokazala je da je RMI veoma pouzdan za diferencijaciju benignih od malignih adneksalnih tumora.

Ključne reči:
jajnik, neoplazme; dijagnoza, diferencijalna; rizik, procena; ultrasonografija; menopauza; histološke tehnike; postoperativni period.

Correspondence to: Milan M. Terzić, Clinical Center of Serbia, Institute of Obstetrics and Gynecology, Koste Todorovića 26, 11 000 Belgrade, Serbia. Phone: +381 11 361 5592; E-mail: terzicmilan@yahoo.co.uk

Introduction

Ovarian cancer is a leading cause of death among gynecological malignant tumors. Treatment efficiency in patients with ovarian cancers could be increased by standardization of preoperative evaluation. Jacobs et al. [1] have introduced risk of malignancy index (RMI) and that was the first diagnostic model which has combined demographic, sonographic and biochemical parameters for investigating patients with adnexal masses. RMI was modified by Tingulstad et al. [2, 3] for the first time in 1996 (RMI2) and for the second time in 1999 (RMI3). These three versions of RMI were assessed in many prospective and retrospective clinical studies. Yamamoto et al. [4] made even RMI4, but its validity is due to be confirmed in future studies. The RMI value of 200 has been proven to be the best for distinction of benign from malignant adnexal masses, with the high level of sensitivity (51%–90%) and specificity (51%–97%) [5, 6].

The aim of the study was to evaluate the risk of malignancy index efficiency in differentiation benign from malignant adnexal tumors in clinical practice.

Methods

The study involved all patients with adnexal tumors who were hospitalized at the Institute of Gynecology and Obstetrics, Clinical Center of Serbia during the first six months of 2010. Ultrasonographic examination of pelvic organs was performed, menopausal status and level of cancer antigen 125 (CA125) were assessed and finally RMI was calculated for all the patients. RMI was calculated using the formula: RMI = U × M × CA125. In the formula U represents the ultrasound index. Multilocular and bilateral tumors, the presence of solid parts in tumor, metastasis and ascites are marked with one point each. The sum of these points, are scored so that in the formula U 0 = 0 points, U 1 = 1 points, U 2–5 = 3 points. In the formula M represents menopausal status (1 for premenopausal and 3 for postmenopausal women). Values of CA125 are taken in all patients prior to surgery. The patients were divided into three groups according to the RMI values (low risk < 25, intermediate risk 25–200, high risk > 200). After surgery, histopathological (HP) findings of excised tumors were analyzed in order to determine the final diagnosis and the stage the disease. Finally, based on the standard formulas, sensitivity [(true positive / true positive + false negative) × 100], specificity [(true negative / true negative + false positive) × 100], positive [(true positive / true positive + false positive) × 100] and negative [(true negative / true negative + false negative) × 100] predictive values of RMI, as a test which could indicate tumor malignancy, were calculated. For statistical analysis of the achieved data standard methods of data description (mean values, variability measures such as confidence interval and standard deviation) as well as the tests for examining the significance of correlations and differences between achieved data (chi- square test, Fisher T-test and Spearman Rank correlation) were used. For statistical data analysis SPSS 15 computer program was used.

Results

The study involved 81 patients, out of which 51 (62.96%) were still in the reproductive period, while 30 (37.04%) were in the menopause. Out of all analyzed patients benign tumor had 51 (62.96%) and malignancy 30 (37.04%) women. In the group of premenopausal women benign tumor was registered in 38 (74.51%) and malignancy in 13 (25.5%) patients. In the group of postmenopausal women benign tumor was registered in 13 (43.33%) and malignant in 17 (56.67%) patients. From all benign tumors ovarian cyst was the most frequent, while from the malignant tumors adenocarcinoma was found to be the most usual (Figure 1). The HP diagnoses of benign adnexal masses are shown in Table 1while Table 2 shows HP diagnoses of malignancy.

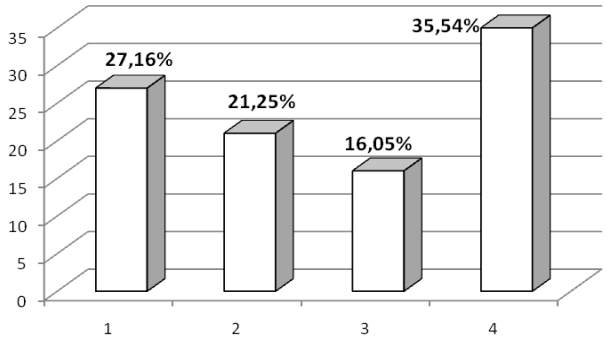


Fig. 1 – Adnexal tumors' histological findings of all the investigated patients

1– benign ovarian cyst; 2 – adenocarcinoma; 3 – endometriosis; 4 –other

The average age of the investigated patients with benign tumors was 38.3 years, whereas, the average age of the investigated patients with malignant tumors was 51.45 years, which was significantly different ($p = 0.001$, $p < 0.05$). The average value of CA125 in the group of patients with benign adnexal tumors was 68.3 U/mL, while in the group of those with malignant tumors it was 581.95 U/mL. However, there were no significant differences in CA125 values concerning categories of HP findings (benign, malignant) ($p = 0.342$, $p > 0.05$). These data are shown in the Table 3.

Multilocular changes with solid tumor components were registered in 5 (6.71%) of the patients who were all premenopausal. Only multilocular changes were registered in 24 (29.63%) of the patients, 14 pre- and 10 postmenopausal. Tumor had only solid parts in 18 (22.22%) patients, 8 pre- and 10 postmenopausal. Metastases were registered in 11 (13.58%) of the patients, out of which 6 were postmenopausal and ascites was registered in 13 (16.05%) of the patients, out of which only 4 were premenopausal. Tumors were present on both ovaries in 13 (16.05%) women, 8 pre- and 5 postmenopausal.

Using the given formula, RMI was calculated for each patient. In the group of patients with benign adnexal masses average RMI was 284.9 (min = 0, max = 2,889), while in the group with malignant changes it was 469.2 (min = 0, max = 122,607). Relation between RMI, HP findings and menopausal status is shown in the Table 4.

Table 1

Histopathological (HP) findings in the patients with benign adnexal tumors

HP diagnosis	Total number (%) of patients	Percentage of benign findings	Patients	
			Premenopausal n (%)	Postmenopausal n (%)
Cystis haemorrhagica ovarii	3 (3.7)	5.88	2 (3.92)	1 (3.24)
Cystis corporis lutei	3 (3.7)	5.88	3 (5.88)	0
Cystis hydatidosa Morgagni	4 (4.94)	7.84	4 (7.85)	0
Cystis symplex ovarii	9 (11.11)	17.65	6 (11.76)	3 (10.00)
Cystis folicularis ovarii	3 (3.7)	5.88	3 (5.88)	0
Cystis endometriotica	13 (16.05)	25.49	10 (19.61)	3 (10.00)
Fibroadenoma ovarii	2 (2.47)	3.92	2 (3.92)	0
Cystadenoma serosum	4 (4.94)	7.84	2 (3.92)	2 (6.67)
Cystadenoma mucinosum	4 (4.94)	7.84	2 (3.92)	2 (6.67)
Cystadenoma mixtus benignum	1 (1.23)	1.96	1 (1.97)	0
Tu Brener benignum	1 (1.23)	1.96	0	1 (3.24)
Teratoma cysticum benignum – cystis dermoidalis bill	4 (4.94)	7.84	3 (5.88)	1 (3.24)

Table 2

Histopathological (HP) findings in the patients with malignant adnexal tumors

HP diagnosis	Total number (%) of patients	Percentage of malignant findings	Patients	
			Premenopausal n (%)	Postmenopausal n (%)
Adeno Ca endometrioides ovar.	5 (6.18)	16.67	2 (3.92)	3 (10.00)
Adeno Ca papillare serosum	4 (4.94)	13.33	1 (1.97)	3 (10.00)
Adeno Ca serosum ovarii	5 (6.18)	16.67	2 (3.92)	3 (10.00)
Adeno Ca slabodiferentovan	4 (4.94)	13.33	2 (3.92)	2 (6.67)
Tu mixtus malignus – CaSa	1 (1.23)	3,33	0	1 (3,24)
Yolk sac tumor ovarii Ic	1 (1.23)	3.33	0	1 (3.24)
Fibrosarcoma ovarii	2 (6.67)	6.67	0	2 (6.67)
Atipicaly proliferating serosus borderline Tu ovarii	4 (4.94)	13.33	3 (5.88)	1 (3.24)
Atipicaly proliferating mucosus borderline Tu ovarii	4 (4.94)	13.33	3 (5.88)	1 (3.24)

Table 3

Age and CA125 levels in the patients with benign and malignant adnexal tumors

Parameters	Benign tumors			Malignant tumors		
	min	max	average	min	max	average
Age (years)	16	72	38.3	16	82	51.45
CA125 (U/mL)	4.2	963	68.3	2.4	13623	581.95

Table 4

Risk of malignancy index (RMI) categories in the investigated patient groups

HP diagnosis	RMI								
	< 25			25–200			> 200		
	Prem n (%)	Postm n (%)	Total n (%)	Prem n (%)	Postm n (%)	Total n (%)	Prem n (%)	Postm n (%)	Total n (%)
BT (n = 51)	26 (50.98%)	5 (16.67%)	31(60.78%)	10 (19.61%)	7 (23.33%)	17 (33.34%)	2 (3.92%)	1 (3.33%)	3 (5.88%)
MT (n = 30)	1 (1.96%)	4 (13.33%)	5 (16.67%)	8 (15.69%)	4 (13.33%)	12 (40%)	4 (7.84%)	9 (30%)	13 (43.33%)
Sum (n = 81)	27 (52.94%)	9 (30%)	36 (44.44%)	18 (35.29%)	11 (36.37%)	29 (35.81%)	6 (11.76%)	10 (33.33%)	16 (19.75%)

HP – histopathology; BT – benign tumors; MT – malignant tumors; Prem – premenopausal; Postm – postmenopausal

Data shown in Table 4 indicate a positive correlation between both categories of HP findings (benign, malignant) and categories of RMI in all the examined women. This correlation shows that with an increase of RMI values, HP findings are more frequently malignant and that the significant cut off value of RMI is 200 ($Ro_{xy} = 0.428$; df = 78; $p = 0.000$; $p < 0.01$).

There were 3 patients with benign tumors and RMI higher than 200. Those were false positive cases. On the other hand, 5 patients with malignant tumors had RMI less than 200. Those were false negative cases. In the group of premenopausal women false positive results were 2 and false negative 1, while in the group of postmenopausal women there was 1 false positive result and 4 false negative results. The most frequently misdiagnosed were borderline tumors (3 out of 8) or low differentiated tumors (1 out of 4) as well as endometriotic cysts (2 out of 13) and benign mucinous cystadenomas (2 out of 8). Therefore, RMI sensitivity is 83.33%, specificity 94.12%, while the positive predictive value is 89.29% and the negative predictive value is 90.57%.

Discussion

The investigated patients from our population more frequently had benign adnexal masses, which were more frequent in premenopausal women. On the other hand, patients who had malignant tumors were significantly older than those with benign masses and therefore, in postmenopausal group malignant tumors were the most usual.

Our study shows that the risk for malignancy of adnexal tumor based on RMI statistically significantly corresponds with postoperatively obtained HP findings. Among the patients we investigated, there were a lot of cases of benign tumors and high CA125 values. It is well known that the levels of CA125 in premenopausal women can vary regarding the phase of menstrual cycle and that the peak values are during menstrual bleeding [7]. Also, entities such as endometriosis and pelvic inflammatory disease can be the reason for high blood levels of CA125 [8]. In our study, out of all benign tumors 25.49% were endometriotic cysts and that lead to the increase of CA125 levels in the group of women with benign adnexal masses.

On the other hand, borderline tumors were sorted in the group of malignant tumors and they were present in 26.66% of the patients with malignant tumors. That might have influenced a bit lower the average values of CA125 in the group of patients with malignant adnexal masses. Nevertheless, according to the data from the literature, values of CA125 are still of high importance in evaluation of adnexal masses, and therefore must not be disregarded [9–13]. Concerning the above mentioned, RMI has shown to be a significant factor in routine clinical practice and therefore we evaluated its efficiency very precisely.

Based on the RMI analysis that we have done, it can be concluded that all aspects of RMI efficiency are satisfactory (high sensitivity, specificity, positive predictive value and negative predictive value), which can indicate significance of RMI in discrimination between benign and malignant adnexal tumors. The results of our study are in correlation with the data from the literature [14, 15].

However, when we compare the investigated parameters, it can be seen that higher values are achieved for specificity and negative predictive value than for sensitivity and positive predictive value. Good specificity means that test will always disregard negative findings, i.e. in our case that benign tumors will always have low RMI values. That is also confirmed by high negative predictive values. Tests with better specificity are more useful in early diagnostic phases and in population screening and RMI calculation and evaluation is used precisely in those cases. If we compare pre- and postmenopausal women, RMI is more sensitive and specific for the premenopausal group. On the other hand, in both groups as well as in all the patients examined together, a higher specificity was observed. On the contrary, a positive predictive value had maximal values in the group of postmenopausal women, which indicates that high RMI of the patients from this group will usually (92.86% cases) really be a sign of malignant adnexal tumor. A bit lower sensitivity indicates that final diagnostics can not be based only on RMI, as there is still a chance that this method does not diagnose the presence of malignant tumor.

Conclusion

Our study confirms that RMI is very reliable for differentiation between benign and malignant adnexal masses.

R E F E R E N C E S

1. *Jacobs I, Oram D, Fairbanks J, Turner J, Frost C, Grudzinskas JG.* A risk of malignancy index incorporating CA 125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer. Br J Obstet Gynaecol 1990; 97(10): 922–9.
2. *Tingulstad S, Hagen B, Skjeldestad FE, Onsrud M, Kiserud T, Halvorsen T*, et al. Evaluation of a risk of malignancy index based on serum CA125, ultrasound findings and menopausal status in the pre-operative diagnosis of pelvic masses. Br J Obstet Gynaecol 1996; 103(8): 826–31.
3. *Tingulstad S, Hagen B,Skjeldestad FE, Halvorsen T, Nustad K, Onsrud M.* The risk-of-malignancy index to evaluate potential ovarian cancers in local hospitals. Obstet Gynecol 1999; 93(3): 448–52.
4. *Yamamoto Y, Yamada R, Oguri H, Maeda N, Fukaya T.* Comparison of four malignancy risk indices in the preoperative evaluation of patients with pelvic masses. Eur J Obstet Gynecol Reprod Biol 2009; 144(2): 163–7.
5. *Raza A, Mould T, Wilson M, Burnell M, Bernhardt L.* Increasing the effectiveness of referral of ovarian masses from cancer unit to cancer center by using a higher referral value of the risk of malignancy index. Int J Gynecol Cancer 2010; 20(4): 552–4.
6. *Moolthiya W, Yuenyao P.* The risk of malignancy index (RMI) in diagnosis of ovarian malignancy. Asian Pac J Cancer Prev 2009; 10(5): 865–8.

7. *Timmerman D, Van Calster B, Jurkovic D, Valentin L, Testa AC, Bernard JP,* et al. Inclusion of CA-125 does not improve mathematical models developed to distinguish between benign and malignant adnexal tumors. J Clin Oncol 2007; 25(27): 4194–200.
8. *Strigini FA, Gadducci A, Del Bravo B, Ferdeghini M, Genazzani A*R. Differential diagnosis of adnexal masses with transvaginal sonography, color flow imaging, and serum CA 125 assay in pre- and postmenopausal women. Gynecol Oncol 1996; 61(1): 68–72.
9. *Clarke SE, Grimshaw R, Rittenberg P, Kieser K, Bentley J.* Risk of malignancy index in the evaluation of patients with adnexal masses. J Obstet Gynaecol Can 2009; 31(5): 440–5.
10. *Harry VN, Narayansingh GV, Parkin DE.* The risk of malignancy index for ovarian tumours in Northeast Scotland--a population based study. Scott Med J 2009; 54(2): 21–3.
11. *Enakpene CA, Omigbodun AO, Goecke TW, Odukogbe AT, Beckmann MW.* Preoperative evaluation and triage of women with suspicious adnexal masses using risk of malignancy index. J Obstet Gynaecol Res 2009; 35(1): 131–8.
12. *Terzic M, Dokic M, Stimec B.* Immature ovarian teratoma in a young girl: very short course and lethal outcome. A case report. Int J Gynecol Cancer 2005; 15(2): 382–4.
13. *Terzic M, Arsenovic N.* The link between ovarian cancer and infertility drugs. Eur J Gynaecol Oncol 2007; 28(2): 160.
14. *Bailey J, Tailor A, Naik R, Lopes A, Godfrey K, Hatem HM,* et al. Risk of malignancy index for referral of ovarian cancer cases to a tertiary center: does it identify the correct cases? Int J Gynecol Cancer 2006; 16(suppl 1): 30–4.
15. *Ulusoy S, Akbayir O, Numanoglu C, Ulusoy N, Odabas E, Gulkilik A.* The risk of malignancy index in discrimination of adnexal masses. Int J Gynaecol Obstet 2007; 96(3): 186–91.

Received on August 23, 2010.
Accepted on September 22, 2010.

ORIGINAL ARTICLE

UDC: 616.31-07:616.724-07
DOI: 10.2298/VSP1107594T

Correlation analysis of craniomandibular index and gothic arch tracing in patients with craniomandibular disorders

Analiza korelacije kraniomandibularnog indeksa i zapisa gotskog luka kod bolesnika sa kraniomandibularnim disfunkcijama

Jelena Todić, Dragoslav Lazić, Radiovoje Radosavljević

University Priština – Kosovska Mitrovica, Clinic of Prosthodontics,
Kosovska Mitrovica, Serbia

Abstract

Background/Aim. Complex etiology and symptomatology of craniomandibular dysfunction make the diagnosing and therapy of this disorder more difficult. The aim of this work was to assess the value of clinical and instrumental functional analyses in diagnosing of this type of disorders. **Methods.** In this study 200 subjects were examined, 15 with temporomandibular joint disorder. They were subjected to clinical functional analysis (Fricton-Shiffman) and instrumental functional analysis by using the method of gothic arch. The parameters of the gothic arch records were analyzed and subsequently compared among the subjects of the observed groups. **Results.** In the examined group of the population 7.5% of them were with craniomandibular dysfunction. The most frequent symptoms were sound in temporomandibular joint, painful sensitivity of the muscles on palpation and lateral turning of the lower jaw while opening the mouth. By analyzing the gothic arch records and comparing the obtained values between the observed groups it was assessed that: lateral and protrusion movements, lateral amplitude and the size of gothic arch were much bigger in the healthy subjects, and latero-lateral asymmetry was larger in the sick subjects. Latero-lateral dislocation of apex was recorded only in the sick subjects with average values of 0.22 ± 0.130 mm. The correlation between the values of Fricton-Shiffman craniomandibular index and the parameters of the gothic arch records and latero-lateral amplitude and dislocation of apex records were established by correlative statistical analysis. **Conclusion.** Functional analysis of orofacial system and instrumental analysis of lower jaw movements (gothic arch method) can be recommended as precise and simple methods in diagnosing craniomandibular dysfunctions.

Key words:
craniofacial abnormalities; mandible; jaw abnormalities; jaw relation record; mobility limitation; sensitivity and specificity; serbia.

Apstrakt

Uvod/Cilj. Složena etiologija i simptomatologija kraniomandibularnih disfunkcija značajno otežavaju dijagnostiku i terapiju ovog poremećaja. Cilj rada bio je da se utvrdi validnost kliničke i instrumentalne funkcijske analize za dijagnostiku ove vrste poremećaja. **Metode.** U ovoj studiji pregledano je 200 ispitanika, kod 15 ustanovljena je kraniomandibularna disfunkcija. Kontrolna grupa formirana je od 15 ispitanika bez znakova i simptoma kraniomandibularne disfunkcije. I oboleli i zdravi ispitanici podvrgnuti su kliničkoj funkcijskoj analizi prema Fricton-Shiffman-u i insrumentalnoj funkcijskoj analizi metodom gotskog luka. Parametri zapisa gotskog luka analizirani su i naknadno upoređeni između ispitanika posmatranih grupa. **Rezultati.** U pregledanom delu populacije kraniomandibularne disfunkcije bile su zastupljene kod 7,5% ispitanika. Simptomi za koje je utvrđena najveća učestalost bili su: zvuk u viličnom zglobu, bolna osetljivost mišića na palpaciju i lateralno skretanje donje vilice u toku otvaranja usta. Analizom zapisa gotskog luka i komparacijom dobijenih vrednosti između posmatranih grupa utvrđeno je da su lateralne i protruzione kretnje, laterolateralna amplituda i veličina gotskog luka značajno veće kod zdravih ispitanika, a da je laterolateralna asimetričnost veća kod obolelih ispitanika. Laterolateralna dislokacija apeksa registrovana je samo kod obolelih ispitanika sa prosečnom vrednošću $0,22 \pm 0,130$ mm. Korelacionom analizom utvrđena je međuzavisnost vrednosti kraniomandibularnog indeksa prema Fricton-Shiffman-u i parametara zapisa gotskog luka, i to laterolateralne amplitude i dislokacije apeksa zapisa. **Zaključak.** Funkcijska analiza orofacijalnog sistema i instrumentalna analiza pokreta donje vilice (metoda gotskog luka) mogu se preporučiti kao precizne i jednostavne metode za dijagnostiku kraniomandibularnih poremećaja.

Ključne reči:
kraniofacijalne anomalije; mandibula; vilice, anomalije; vilice, odnosi; pokretljivost; osetljivost i specifičnost; srbija.

Correspondence to: Jelena Todić, University Priština – Kosovska Mitrovica, Clinical of Prosthodontics, Kosovska Mitrovica, Serbia. Phone: +381 34 345 476. E-mail: todic.j@gmail.com

Introduction

Orofacial system is a set of morphologically and functionally different tissues and organs integrated by means of a neuromuscular connection.

Disruption of the function of some of the orofacial system structures will lead to repercussion over the remaining components, which often results in dysfunction with a complex symptomatology. Symptoms that may appear are joint, dental and muscular in nature. A set of these symptoms has been consolidated under the name of craniomandibular disorders (CMD) [1, 2].

By means of an epidemiological study it has been determined that 50%–75% of people in the course of life experience some of the transient symptoms of joint disease. About 33% have at least one symptom, *eg* pain in the face area or pain in the temporomandibular joint (TMJ). However, only 5% of patients seek medical assistance [1, 3–19]. It was further established that the highest prevalence of the disease is evident in patients between 15 and 45 years of age. This disease is more common in women than in men whereas the ratio is 3 : 1 to 9 : 1 [1, 6, 15].

The complex etiology and craniomandibular dysfunction symptoms significantly complicate diagnosis and treatment of this disease. Adequate approach, and systematic use of numerous recognized methods have become indispensable principles of modern diagnostic dysfunction.

An integral part in craniomandibular dysfunction diagnosis refers to clinical functional analysis. Clinical examination of orofacial structure has been simplified by means of a specially formulated questionnaire, which in addition to its diagnostic value, has a great significance in monitoring the incidence of this disorder as well as the representation of its signs and symptoms [2, 4].

Within dysfunction diagnosis, in addition to clinical functional analysis, an instrumental functional analysis is often conducted. Nowadays, a wide variety of different instrumental methods for the registration of mandibular movements is available. A great part of these methods is based on the presentation of motion in the form of a mechanical tracing on a certain material. Transformation of kinetic movement in a static record is inevitably accompanied by a loss of precision. Nevertheless, these methods provide a simple overview of the muscle-joint system condition and facilitate the diagnosis of CMD. One of the methods of instrumental functional analysis is the intraoral gothic arch tracing. The gothic arch record and normal values of its individual parameters, compared to the values of the same parameters registered with affected subjects, *ie* the patients, represent the starting point for identifying joint disruptions. Therefore, the aim of this study was to determine the presence of CMD in the screened part of the population by means of Fricton-Shiffman clinical functional analysis, and determine the severity of dysfunctional disorders (Craniomandibular Index) in patients; to compare features of the gothic arch record registered in the healthy subjects (control group) to the ones registered in the patients with craniomandibular disorders (experimental group), and to determine the correlation of changes in the gothic arch record and the craniomandibular index values within the experimental group.

Methods

The first phase of the research included 200 randomly chosen subjects from the northern part of Kosovska Mitrovica. The present clinical trial was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Ethical Committee of Kosovska Mitrovica.

The subjects were subjected to the Fricton-Shiffman clinical functional analysis and craniomandibular index determination. This analysis includes: medical history, functional analysis of mandibular movement, sound detection in the temporomandibular joint, palpation of masticatory and neck muscles and palpation of temporomandibular joints. The results of the clinical functional analysis were expressed numerically (0 or 1) and the values were further used to calculate the craniomandibular index (CMI).

Functional analysis of the mandibular movement included testing of mouth opening and closing movements, laterotrusional and propulsion movements of the lower jaw. The results of functional analysis were registered in a specially formulated questionnaire and expressed in terms of numerical values. Positive findings indicating the presence of disorder were scored as 1, whereas negative findings indicating the absence of disorder and normal function of the orofacial system, were scored as 0. The sum of all points obtained by mandibular movement analysis is referred to as the Mandibular Mobility (MM).

Sound signals in TMJ may be registered in various stages of mandibular movement and are expressed in terms of a "click" or a crepitus. The presence of a sound signal was scored as 1, whereas the absence was scored as 0. Points obtained by joint sound signal recording were summed and referred to as the TMJ sound (TMJS).

Palpation of masticatory muscles was implemented as extraoral and intraoral palpation. Extraoral palpation implied examination of: *m. temporalis*, *m. masseter*, *m. pterygoideus medialis*. Intraoral palpation examination covered: *m. pterygoideus lateralis, m. pterygoideus medialis* and a lower insertion of the *m. temporal*. Temporal muscle is palpated extraorally and intraorally. Intraoral palpation is performed by sliding a finger over anterior ramus up to the coronoid notch area, whereas extraoral one refers to the area of its attachment to the temporal bone. The muscle *pterigoideus medialis* is palpated bi-manually. Forefinger of one hand is used to palpate the inner part of mandible angulus, while the other one is used simultaneously for extraoral palpation of the same region. The muscle *pterygoideus lateralis* is quite inaccessible to palpation. Palpation is performed intraorally, placing the tip of the little finger inside the patient's mouth at the moment when he/she opens his/her mouth and moves the lower jaw to the side of the muscle being tested. It is recommended that a patient simultaneously moves the lower jaw upward while a therapist is palpating the region behind the tuber of the upper jaw. The points obtained by extraoral palpation of the muscles on both the left and the right side were

summed and the sum obtained is referred to as extraoral palpation (EP), whereas the sum of the points obtained by intraoral palpation is referred to as intraoral palpation (IP). Palpation of the neck muscles included *m. sternocleidomastoideus* and *m. trapesis*. The points were summed and referred to as the palpation of the neck muscles (PNM).

Palpation of the TMJ capsule included palpation of the upper, side and rear part of the capsule. Points obtained by palpation were summed and referred to as the TMJ capsule palpation (TMJCP).

Craniomandibular index has been obtained by means of dysfunction index (DI) [DI = (+ MM + TMJS TMJCP) / 26)] and palpation index (PI) [PI = (EP + IP + PNM) / 36] by means of summing the obtained values of these indexes and dividing the sum by 2 [CMI = (DI + PI) / 2]. Craniomandibular index is a numerically expressed degree of disease providing a clear insight into the orofacial system condition. Normal values of this index range from 0 to 1.

The results of the Fricton-Shiffman clinical functional analysis conducted in 15 out of 200 patients indicated that signs and symptoms attributed to CMD were registered accordingly, *ie* CMI in these patients was greater than 0. These patients were not subjected to orthodontic and prosthetic treatment. They were assorted into the experimental group during further investigation. The control group was formed out of 15 patients who had no signs and symptoms of craniomandibular dysfunction. These patients had their dental arches preserved, stable intercuspal position and were not treated orthodontically.

The second phase of this study included registration of mandibular movements in horizontal plane by means of an intraoral method, applied to the patients assorted into the experimental and control group (Gothic Arch Method). Registration system we used implies a tracer consisting of a treaded pin and a flat metal contact plate fixed to individually crafted acrylic trays. The tracer consists of a metal casing and a screw with threads bolted to the casing. Top screw is conical with a slightly rounded tip enabling tracing on a metal plate upon mandibular movements. Owing to the threads, the threaded pin length can be controlled and adjusted, and therefore regulate the level of teeth disocclusion. The shape of the contact plate is trapezoidal, 32 mm wide. Production of acrylic trays was preceeded by preliminary upper and lower jaw impression, model casting and their preparation (filling undercuts). The trays are made of autopolymerizing acrylic resins (Simgal).

A top tray covered the palatum, anterior cingulum and palatal surfaces of posterior teeth all the way to the equator, completing the palatal aspect of interdental space, which ensured its retention and stability during the registration process. The upper tray had an embedded casing placed in a position that is perpendicular to the occlusal plane. In addition, the casing was secured at the intersection points of the sagittal midline and the line connecting the mesial surface of maxillary first molars (Figure 1). A bottom tray covered the lingual part of the alveolar bone of the lower jaw, the lingual surface of lower front teeth, up to 1 mm, below the incisal edge, and the lingual cusps of lower side teeth. The lower acrylic tray was fixed to a metal plate parallel to the occlusal plane of the lower jaw (Figure 1). A layer of wax in color was applied onto the metal plate in order to make the tracing visible, whereas the threaded pin range was set up to provide teeth disocclusion of at least 2 mm.

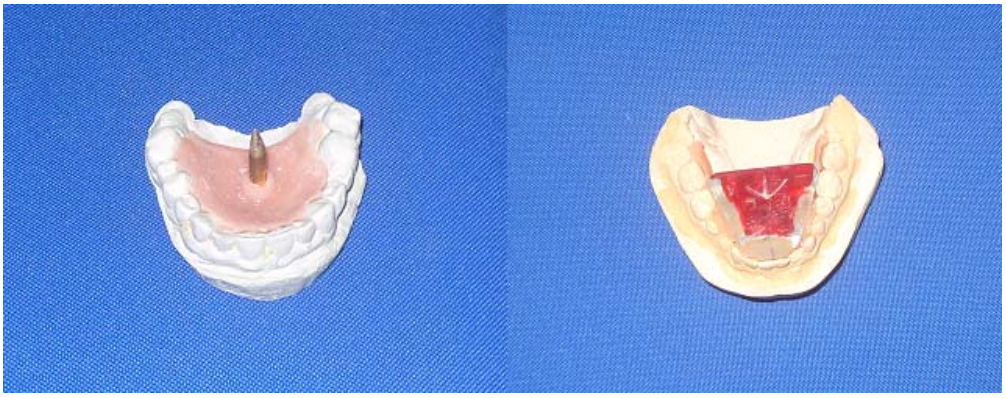


Fig. 1 – System for registration of the gothic arch

The very registration process implied placing acrylic trays in their deposits in the mouth and bringing the lower jaw to the position of central relation by means of a bimanual manipulation according to Dowson [5]. This method involves semi-lying position of patients with the head tilted back in order to prevent the mechanical protrusion of the lower jaw on manipulation. The therapist places his left and right hand fingers on the lower edge of the mandible, placing the thumbs on the chin of a patient. By means of light manipulation, the therapist moves the mandible up and down until it begins to rotate freely, and then he directs it downwards, backwards and upwards for the condyles to reach the highest position in the articulator fossae.

When the lower jaw gets into the central relation position, the threaded pin tip touches the contact plate enabling teeth disocclusion by its height (Figure 2). Then, a subject is required to advance the lower jaw forward as far as possible, and then backwards likewise. The movements are repeated several times. Upon registration of propulsion movements, the registration of lateral move-

ments is performed in exactly the same way. Namely, the subject advanced the lower jaw from the initial rest position moving it up as far as possible to one side and back, and then to the other side and back to the central relation position.

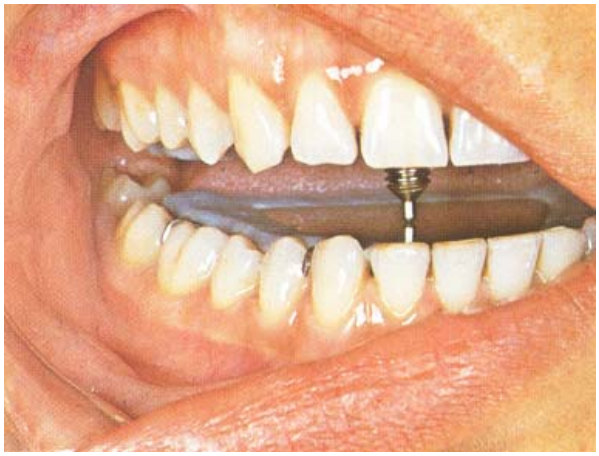


Fig. 2 – Gothic arch registration procedure

Analysis of the obtained results was carried out within a coordinate system (composed on a sheet of graph paper), which is defined by means of four reference points. The first point was located behind the contact point of the lower central incisors. The second point was located at the centerline of the angle, formed by the left and the right tracing arms. The third and the fourth points, at the endpoints of the record arms, were obtained by means of drawing a line on the left and the right side accordingly, right-angled to the respective vertical reference axis passing through the left, *ie* the right endpoint of the gothic arch tracing arms (Figure 3).

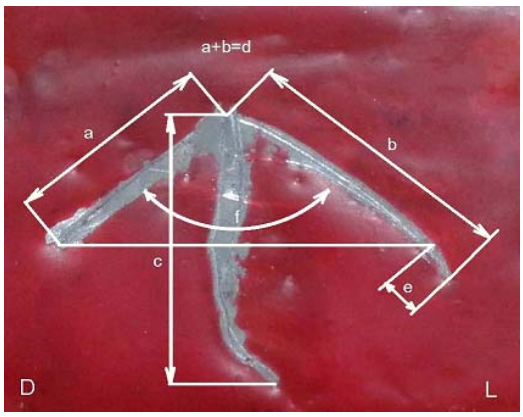


Fig. 4 – Gothic arch record parameters
a – record length of the right lateral movement; b – record length of the left lateral movement;
c – record length of the protrusion movement; a+b = d – lateral movement amplitude;
e – laterolateral asymmetry; f – angle of the gothic arch record

Results

During this study, 200 subjects underwent the Fricton-Shiffman clinical function analysis, with the results indicating dysfunctional disorders registered in 15 subjects, *ie* 7.5% of the treated population. The subjects with dysfunctional changes had their CMI determined, where the lowest index value was 0.04 and the highest 0.360. The average value of CMI in the patients was 0.177 ± 0.106. Out of 15 subjects with signs and symptoms attributed to CMD, 9 subjects were female (60%) whereas the number of male subjects was 6 (40%). Average age of the experimental group subjects was 22.4 ± 6.8 years, whereas that of the control group ones implied 25.7 ± 4.0 years.

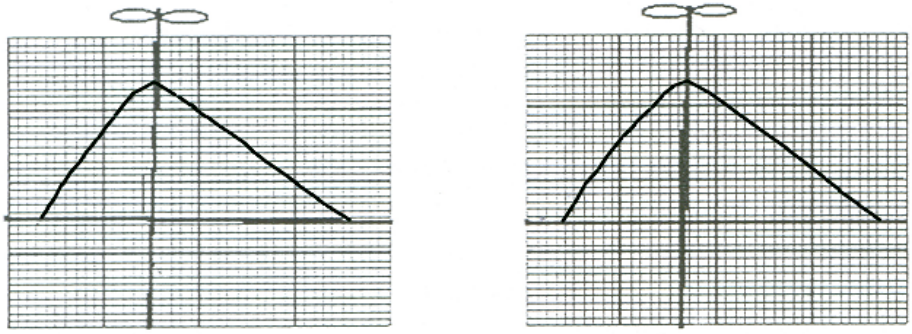


Fig. 3 – Formed coordinate system for measuring parameters of gothic arch

The parameters that were measured and subsequently compared among the subjects within the experimental and control groups imply: length of gothic arch tracing arms and average protrusive tracking length, total length of tracing record arms (lateral movement ampitudes), the difference in the length of record arms (laterolateral asymmetry), the angle formed by the left and the right arm of lateral movement records (gothic arch), discrepancy in the gothic arch apex in relation to the vertical reference coordinate system axis (laterolateral dislocation of the gothic arch apex) (Figure 4).

The functional analysis conducted thereby helped us collect and systematize the signs and symptoms of craniomandibular dysfunction in the examined patients. It was determined that symptoms of acoustic signals during mandibular movement, mandibular deflection to the affected side on mouth opening and muscle tenderness to palpation were the most common ones (Table 1).

The first characteristic of the gothic arch record examined referred to the records of the right and left lateral movements, registered in the groups of subjects undergoing

Table 1

Distribution of signs and symptoms of craniomandibular disorders (CMD) in subjects of the experimental group (EG)

Symptoms and sings of CMD	EG	
	n	%
Limited mouth opening	6	40
Muscle sensitivity on palpation	10	66.6
Deviation of lower jaw during opening mouth	10	66.6
Joint sound	12	80
Skipping during opening mouth	1	6.6
Opening the mouth outside normal limits	2	13.3
Clinically apparent dislocation of condyle	1	6.6

***$p \leq 0.05$ (Student's *t*-test)**

the trial. The obtained length values within right and left lateral movement records were analyzed and statistically compared (Table 2).

Using *t*-test for the purpose of making a comparison between the average length values within the right lateral movement record in the subjects of the studied groups (t = -7.876, p = 0.001) and the left lateral movements in the subjects of the studied groups (t = -3.276, p = 0.003), we found a statistically significant difference between the compared values. This would mean that the length values within right and left lateral movement records were significantly higher in the control group than they were in the experimental group.

Comparison of average length values of the protrusive records, obtained in the experimental and control group subjects (t = -3.809, p = 0.001) showed a statistically significant difference, *ie* average value of this parameter was significantly higher in the control group subjects (Table 3).

The total length of left and right lateral movement records represents the amplitude of laterolateral movements. Statistical analysis showed that there were significant differences in mean amplitude between the experimental and control group subjects (t = -7.656, p = 0.00), *ie* that the average length of laterolateral movements increased in the control group. The difference in the left and right lateral movement records of the experimental and control groups was represented by the lateral asymmetry. Statistical analysis showed that there was a significant difference in mean lateral asymmetry between the experimental and control groups (t = 4.48, p = 0.001). This shows that the average value of this parameter increased in the experimental group compared to the control group (Table 4).

The angle formed by the left and right laterotrusion movement records is known as the gothic arch. Statistical analysis showed significant difference in the size of the angle between the groups (t = -2.160, p = 0.04), *ie* the average value of the gothic arch in the control group was higher than the average value in the experimental group (Table 5).

Lateral dislocation of the gothic arch record apex was registered only in the experimental group, as left or right dislocation. Dislocation was measured in relation to vertical reference axis of the coordinate system. The average value of laterolateral dislocation of the apex record was 0.22 ± 0.13 mm (range : 0.05–0.5 mm).

Table 2

Record of the lateral movements in subjects examined

Subjects	Lateral movement (mm), $\bar{x}$ ± SD (min–max)	
	right	left
Experimental group (n = 15)	8.27 ± 1.03 (7–10)	8.16 ± 2.8 (5–13)
Control group (n = 15)	11.4 ± 1.19* (10–14)	10.66 ± 1.23* (9–13)

***$p \leq 0.05$ (Student's *t*-test)**

Table 3

Record of the protrusion movements in subjects examined

Subjects	Lateral movement (mm) $\bar{x}$ ± SD (min–max)
Experimental group (n = 15)	8.93 ± 1.58 (7–12)
Control group (n = 15)	11.06 ± 1.49* (8–14)

***$p \leq 0.05$ (Student's *t*-test)**

Table 4

Lateral movement amplitudes (LLAm) and laterolateral asymmetry (LLAs) in subjects examined

Subjects	LLAm (mm)	LLAs (mm)
	$\bar{x}$ ± SD (min–max)	$\bar{x}$ ± SD (min–max)
Experimental group (n = 15)	15.8 ± 2.65 (12–21)	2.8 ± 1.32 (0–4)
Control group (n = 15)	22.2 ± 1.86* (19–25)	1.07 ± 0.7* (0–2)

***$p \leq 0.05$ (Student's *t*-test)**

Table 5

Gothic arch in subjects examined

Subjects	Gothic arch (°)
	$\bar{x}$ ± SD (min–max)
Experimental group (n = 15)	10.6 ± 9.61 (91–125)
Control group (n = 15)	116.7 ± 15.4* (94–142)

***$p \leq 0.05$ (Student's *t*-test)**

Table 6

Correlation between craniomandibular index (CMI) and parameters of the gothic arch record

CMI	LL asymetry	LL amplitude	Apex dyslocation
Value of correlation test	r = -0.236	r = -0.586	r = -0.907
Statistical significance (*p*)	> 0.05	< 0.05	< 0.05

LL – latero-lateral

Craniomandibular index is a numerically expressed degree of the orofacial system disease. By means of the Pearson correlation analysis we tried to determine how changes in the Gothic arch record traces within experimental group affect the value of the CMI and *vice versa*, how changing the value of CMI affects the values of the gothic arch record parameters. Values of certain parameters of the gothic arch record were tested in relation to the CMI value (Table 6).

It was determined that there was a correlation between the laterolateral amplitude and the CMI (r = -0.586, p = 0.022) indicating that the decline in value of laterolateral amplitude increases the value of CMI which is shown graphically (Figure 5).

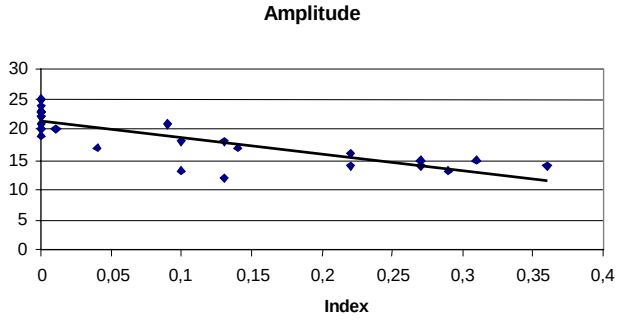


Fig. 5 – Correlation of lateral movement amplitude and craniomandibular index

It was also determined that there is a correlation between the CMI and the laterolateral dislocation of the gothic arch apex (r = -0.907, p = 0.00) indicating that the increase in the value of CMI increases the gothic arch apex dislocation (Figure 6).

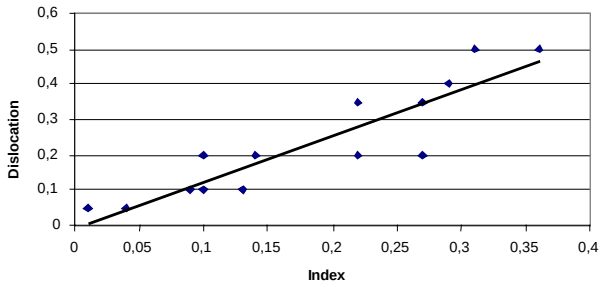


Fig. 6 – Correlation of apex records dislocation and craniomandibular index

Discussion

Craniomandibular dysfunctions stand for disturbances in the function of the orofacial system, multicausal etiology, acute and chronic pathogenesis. One of the persistent problems associated with this type of disorder is certainly its frequency. Our research revealed that dysfunctional disorders occurred in 7.5% of the population screened thereby. List et al. [12] found an identical incidence of CMD in one region of Sweden, whereas Schmitter et al. [13] identified the frequency of 9.93% in the territory of Germany. The study by Dodić et al. [14] indicates that, in the territory of Serbia, the frequency of CMD amounts to 15%. Reports of foreign studies are multifarious. Some studies suggest low prevalence of this disorder (5%) [6–8, 11], unlike the others that point to its extremely high frequency (50%) [9, 10].

Such contradictory data arising from a number of analogue studies can be explained by differences in the number of subjects covered by the study, different methods used for data collection, for evaluation of individual symptoms and the very characteristics of the screened population (socioeconomic status, age and gender of the subjects involved).

The ratio in terms of gender in the patient group was 2.5 : 1 in favor of women. Similar epidemiological studies reported that this disease is more frequent in women than in men [1, 6, 15, 16]. The mechanisms underlying sex differences in prevalence of craniomandibular dysfunctions are still unclear and likely to include physiological and psychosocial factors. Gender differences in the occurrence of muscle tenderness, as the dominant symptom of craniomandibular dysfunctions, can be partially explained by the impact of the female sex hormone estrogen which appears to alter the excitability on afferent fibers and sensory trigeminal neurons, which changes their excitation in conditions of harmful tissue stimulation [17, 18].

Clinical functional analysis showed that the most common symptoms of dysfunctional disorders imply: TMJS, palpable muscle tenderness and mandibular deformation on mouth opening. Gesch et al. [10] and Dodić et al. [14] also find a wide representation of these symptoms in patients with craniomandibular dysfunctions, while Cooper and Kleinberg [19] assign headache to the dominant symptom class as well.

Changes in the gothic arch records in patients in relation to the same records in healthy subjects tend to be con-

firmed by differences in the values of some of its parameters. Data analysis showed a significant difference in the length values of the right and left lateral movement records between the experimental and control groups, *ie* the average value of this parameter was significantly higher in the control group. Similar results have been presented by other authors as well [20–22]. These findings may be supported and explained by a condyle-disc joint complex derangement and muscle function disorder. Uncoordinated activity between condyle and *discus articularis* does not lead to serious mechanical obstructions in the joint, especially when it comes to *discus articularis anterior* dislocation with reduction. However, inadequate position of condyles in bilaminar zone of *discus articularis* causes pain and discomfort, which consequently can lead to muscle spasms, which inevitably leads to the reduction in the range of lateral movements. Besides, the inadequate function of condyle-disc complex may appear due to *discus articularis* dislocation without reduction, when lateral movement is limited due to a blockade in translational movement of the joint.

Dodić et al. [23], Nielsen et al. [24], Obrez and Stobler [25] point out that the difference in the length of lateral movements in subjects with and without dysfunctional disorder amounts to 3–5 mm on average.

Statistical analysis demonstrated a significant difference in the length values of protrusion movement records between experimental and control groups, with the average value amounting to 2 mm. Analogous studies conducted by various authors report similar results [23, 25]. Nielsen et al. [24] do not come up with a statistically significant difference in the length values of protrusion movement records between subjects with and those without dysfunctions. He points out that "only subjects with muscle pain can demonstrate asymmetric laterotrusion with a protrusion projecting normal pathway".

Laterolateral amplitude is also one of the characteristics of the gothic arch tracing record that may indicate the presence of dysfunctional disorders. Processing analysis data showed significant differences in mean amplitude of lateral movements between experimental and control group subjects, *ie* it was significantly higher in healthy subjects [26]. Comparative analysis of average values of laterolateral asymmetry between the groups is statistically significant, *ie* it indicates that the average value of this parameter increased in the experimental group compared to the control group. Okeson [27] and Peters and Gross [28] point out that the average value of the difference in the length of left and right lateral movement records in healthy subjects is less than 2 mm and also, that higher laterolateral asymmetry indicates the presence of dysfunctional disorders. The angle formed by the left and the right lateral movement records is called the 'gothic arch'. By means of the statistical analysis we found that there were significant differences in the values of the gothic arch registered in the experimental and control groups, and that the average value of this parameter is significantly higher in healthy subjects.

Correlation analysis indicated that the parameters of apex dislocation and lateral amplitude change synchronously with alterations in values of CMI. Specifically, the analysis showed that increase in apex dislocation causes increase in the value of the CMI and *vice versa*. Statistical association points out to the fact that the decline in the value of lateral amplitude is followed by an increase in the value of CMI.

Conclusion

The application of Fricton-Shiffman clinical functional analysis leads into the conclusion that the frequency of craniomandibular function incidence in the territory of the northern part of Kosovska Mitrovica amounts to 7.5%, and that a mild degree disorders have been registered accordingly.

The gothic arch record in patients showed a significant change in its parameters in comparison with the one registered in healthy subjects.

The degree of these changes in the Gothic arch records depends on the degree of dysfunctional disorder, *ie* CMI values. This indicates that these two stand for two mutually different methods of similar sensitivity in terms of identification of dysfunctional disorders, and that a combined approach can be used in the diagnostic protocol of this type of disorder. Therefore, the functional analysis of the orofacial system and the instrumental analysis of the mandibular movement (Gothic Arch Method) can be recommended as accurate and simple methods in craniomandibular disorder diagnosis.

REFERENCES

1. *McNeill C, Bell WE, Delaat A, Dworkin SF.* Craniomandibular disorders. Guidelines for evaluation, diagnosis and management. Chicago: Quintessence Publishing Co; 1990.
2. *Fricton JR, Schiffman EL.* Reliability of a craniomandibular index. J Dent Res 1986; 65(11): 1359–64.
3. *Stanišić D, Krstić M, Miljković Ž, Šašić M, Glišić B, Marković LJ,* et al. Craniomandibular joint – normal function and disorders. Belgrade: BMG – Beogradsko mašinsko-grafičko preduzeće; 2001. p. 61–85. (Serbian)
4. *Helkimo M.* Studies on function and dysfunction of the masticatory system. II. Index for anamnestic and clinical dysfunction and occlusal state . Swed Dent J 1974; 67(2): 101–21.
5. *Dawson PE.* Evaluation, diagnosis and treatment of occlusal problems. 2nd ed. St. Louis, MO: Mosby; 1989. p. 1–13.
6. *Macfarlane TV, Blinkhorn AS, Davies RM, Kincey J, Worthington HV.* Oro-facial pain in the community: prevalence and associated impact. Community Dent Oral Epidemiol 2002; 30(1): 52–60.
7. *Isong U, Gansky SA, Plesh O.* Temporomandibular joint and muscle disorder-type pain in U.S. adults: the National Health Interview Survey. J Orofac Pain 2008; 22(4): 317–22.
8. *Nilsson IM, List T, Drangsholt M.* Incidence and temporal patterns of temporomandibular disorder pain among Swedish adolescents. J Orofac Pain 2007; 21(2): 127–32.
9. *Casanova-Rosado JF, Medina-Solís CE, Vallejos-Sánchez AA, Casanova-Rosado AJ, Hernández-Prado B, Avila-Burgos L.* Prevalence and associated factors for temporomandibular disorders in a group of Mexican adolescents and youth adults. Clin Oral Investig 2006; 10(1): 42–9.

10. *Gesch D, Bernhardt O, Alte D, Schwahn C, Kocher T, John U,* et al. Prevalence of signs and symptoms of temporomandibular disorders in an urban and rural German population: results of a population-based Study of Health in Pomerania. Quintessence Int 2004; 35(2): 143–50.
11. *Magnusson T, Egermark I, Carlsson GE.* A longitudinal epidemiologic study of signs and symptoms of temporomandibular disorders from 15 to 35 years of age. J Orofac Pain 2000; 14(4): 310–9.
12. *List T, Wahlund K, Wenneberg B, Dworkin SF.* TMD in children and adolescents: prevalence of pain, gender differences, and perceived treatment need. J Orofac Pain 1999; 13(1): 9–20.
13. *Schmitter M, Balke Z, Hassel A, Ohlmann B, Rammelsberg P.* The prevalence of myofascial pain and its association with occlusal factors in a threshold country non-patient population. Clin Oral Investig 2007; 11(3): 277–81.
14. *Dodić S, Stanisić-Sinobad D, Vukadinović M, Milić A, Sinobad V.* The prevalence of craniomandibular disorders in the military population of the Republic of Serbia. Med Pregl 2006; 59(5–6): 259–64. (Serbian)
15. *Marklund S, Wänman A.* Incidence and prevalence of myofascial pain in the jaw-face region. A one-year prospective study on dental students. Acta Odontol Scand 2008; 66(2): 113–21.
16. *Janal MN, Raphael KG, Nayak S, Klausner J.* Prevalence of myofascial temporomandibular disorder in US community women. J Oral Rehabil 2008; 35(11): 801–9.
17. *Cairns BE.* The influence of gender and sex steroids on craniofacial nociception. Headache 2007; 47(2): 319–24.
18. *Dao TT, LeResche L.* Gender differences in pain. J Orofac Pain 2000; 14(3): 169–84.
19. *Cooper BC, Kleinberg I.* Examination of a large patient population for the presence of symptoms and signs of temporomandibular disorders. Cranio 2007; 25(2): 114–26.
20. *Bonjardim LR, Gavião MB, Pereira LJ, Castelo PM, Garcia RC.* Signs and symptoms of temporomandibular disorders in adolescents. Braz Oral Res 2005; 19(2): 93–8.
21. *Arutiunov SD, Khvatov IL, Arutiunov DS, Nabiev NV, Tuturov NS.* Diagnosis of the temporomandibular joint dysfunction by graphic reconstruction of mandibular movements. Stomatologiia (Mosk) 2003; 82(1): 27–33. (Russian)
22. *Maloney GE, Mehta N, Forgione AG, Zawawi KH, Al-Badawi EA, Driscoll SE.* Effect of a passive jaw motion device on pain and range of motion in TMD patients not responding to flat plane intraoral appliances. Cranio 2002; 20(1): 55–66.
23. *Dodić S, Sinobad V, Obradović-Đuričić K, Medić V.* The role of occlusal factor in the etiology of temporomandibular dysfunction. Srp Arh Celok Lek 2009; 137(11–12) 613–8.
24. *Nielsen IL, Marcel T, Chun D, Miller AJ.* Patterns of mandibular movements in subjects with craniomandibular disorders. J Prosthet Dent 1990; 63(2): 202–17.
25. *Obrez A, Stohler CS.* Jaw muscle pain and its effect on gothic arch tracings. J Prosthet Dent 1996; 75(4): 393–8.
26. *Lazić V.* Mandibular movements analysis in patients with craniomandibular disorders before and after stabilization therapy with the Michigan Bite Splint [thesis]. Belgrade: Stomatološki fakultet;1988. (Serbian)
27. *Okeson JP.* Management of temporomandibular disorders and occlusion. 4th ed. St. Louis: Mosby; 1998.
28. *Pertes RA, Gross SG.* Clinical management of temporomandibular disorders and orofacial pain. Chicago, IL: Quintessence Publishing; 1995.

Received on December 10, 2009.
Revised on February 2, 2010.
Accepted on February 11, 2010.

CASE REPORTS

UDC: 616.37-002.2:616.37-003.94-06-089
DOI: 10.2298/VSP1107602M

Splenic artery pseudoaneurysm as a complication of pancreatic pseudocyst

Pseudoaneurizma slezinske arterije kao komplikacija pseudociste pankreasa

Saša Micković*, Miroslav Mitrović*, Nebojša Stanković*, Mihailo Bezmarević*, Milan Jovanović*, Darko Mirković*, Ivana Tufegdžić†, Irena Nikolić Micković‡

Military Medical Academy, *Clinic for Abdominal and Endocrine Surgery, †Institute for Pathology; ‡ **University Children's Hospital, Belgrade**, **Belgrade, Serbia**

Abstract

Introduction. Pancreatic pseudocyst presented as pseudoaneurysm of the splenic artery is a potential serious complication in patients with chronic pancreatitis. **Case report.** A 42-year-old male patient with a long-standing evolution of chronic pancreatitis and 8-year long evolution of pancreas pseudocyst was referred to the Military Medical Academy, Belgrade due to worsening of the general condition. At admission, the patient was cachectic, febrile, and had the increased values of amylases in urine and sedimentation (SE). After clinical and diagnostic examination: laboratory assessment, esophagogastroduodenoscopy (EGDS), ultrasonography (US), endoscopic ultrasonography (EUS), multislice computed scanner (MSCT) angiography, pseudoaneurysm was found caused by the conversion of pseudocyst on the basis of chronic pancreatitis. The patient was operated on after founding pancreatic pseudocyst, which caused erosion of the splenic artery and their mutual communication. Postoperative course was duly preceded without complications with one year follow-up. **Conclusion.** Angiography is the most reliable and the safest method for diagnosing hemorrhagic pseudocysts when they clinically present as pseudoaneurysms. A potentially dangerous complication in the presented case was treated surgically with excellent postoperative results.

Key words:
pancreatitis, chronic; pancreatic pseudocyst; aneurysm, false; hypertension, portal; splenic artery; splenic vein; venous thrombosis; digestive system surgical procedures; diagnosis; treatment outcome.

Apstrakt

Uvod. Pankreasna pseudocista koja se prezentuje kao pseudoaneurizma slezinske arterije je potencijalno opasna komplikacija kod bolesnika sa hroničnim pankreatitisom. **Prikaz bolesnika**. Bolesnik, star 42 godine, sa dugogodišnjim hroničnim pankreatitisom i osmogodišnjom evolucijom pseudociste pankreasa primljen je u Vojnomedicinsku akademiju, Beograd zbog pogoršanja opšteg stanja. Na prijemu, bolesnik je bio kahektičan, febrilan, sa povišenom vrednošću amilaze u urinu i povišenom sedimentacijom (SE). Nakon kliničke i dijagnostičke evaluacije: laboratorijski nalazi, ezofagogastroduodenoskopija (EGDS), ultrazvučni pregled (US) abdomena, endoskopski ulztrazvučni (EUS) pregled, multislajsna skenerska (MSCT) angiografija, nađena je pseudoaneurizma koja je nastala konverzijom pseudociste na bazi hroničnog pankreatitisa. Bolesnik je operisan kada je nađena pseudocista pankreasa koja je dovela do erozije slezinske arterije i njihove međusobne komunikacije. Postoperativni tok prošao je uredno, sa praćenjem bolesnika u narednih godinu dana bez komplikacija. **Zaključak.** Angiografija je najpouzdanija i najsigurnija metoda za dijagnostikovanje hemoragične pseudociste, koja se klinički prezentuje kao pseudoaneurizma. Kod prikazanog bolesnika potencijalno opasna komplikacija lečena je hirurški sa odličnim postoperativnim rezultatom.

Ključne reči:
pankreatitis, hronični; pankreas, pseudocista; pseudoaneurizma; hipertenzija, portalna; a. splenica; v. splenica; tromboza, venska; hirurgija digestivnog sistema, procedure; dijagnoza; lečenje, ishod.

Introduction

In recent decades, pancreatic pseudocyst and complications which accompany it [1–2] have been successfully treated using conservative treatment without surgery [3–5]. There are clear adventages of new techniques development and modern achievements over the conventional surgical menagement, but only in clearly defined indications with acceptable risk of complications [6]. However, in cases with the pseudocyst additionally complicated with hemorrhage [1, 2], in the literature

Correspondence to: Saša Micković, Military Medical Akademy, Clinic for Abdominal and Endocrine Surgery, Crnotravska 17, 11000 Belgrade, Serbia. Phone: +381 11 216 40 30, +381 64 401 65 93. E-mail: mcsachanet@yahoo.com

the state known as conversion of pseudocyst in pseudoaneurism, and evolution of regional left-sided portal hypertension (LPH), surgery takes place as the most important method for treatment of this serious complication [7–9]. One of a few surgical procedures that may be a method of choice in hemorrhage and/or rupture of cysts and/or pseudoaneurysms is distal spleno–pancreatectomy [1, 2, 9].

Case report

A patient, at the age of 42, was admitted to the Clinic for Gastroenterology, Military Medical Academy, Belgrade, due to chronic pseudocyst in pancreatic tail region for eight years. Lately, the patient had attacks of pain followed by nausea and vomiting. In the last 15 years the patient consumed alcoholic drinks regularly and in several occasions he had attacks of acute pancreatitis. On admission, he had abdominal pain without jaundice, febrile to 38.5°C. Objective findings were palpable and painful tumefaction and rough systolic auscultation with murmur in the region of epigastrium. Pancytopenia dominated in laboratory status with an emphasis on the fall of platelets (PLT) of 50×10^9/L, sedimentation (SE) 79 mm/Lh, international normalized ratio (INR) 1, and the value of urinary amylase of 1,129 IU/L. Esophagogastroduodenoscopy (EGDS) verified the existence of outside pressure on the fundus area and back wall of the stomach body. Ultrasonography (US) of the abdomen showed the presence of massive calcification, particularly in the area of the pancreatic tail with the existence of pulsating tumefaction with thickened wall in the area of the tail of pancreas. Endoscopic ultrasonography (EUS) showed collection of fluid in the *bursa omentalis*, close to the region of the tail of the pancreas and in the exact region there was a large partially septated collection with flow. Multislice computed tomography (MSCT) angiography of the portal basin showed the number of calcification in the pancreas, enlarged spleen and thrombosis of the splenic vein. Drainage of the spleen was made through the well-developed collaterals around cardio and fundus region of the gizzard and the network of blood vessels which partially went through the frontal abdominal wall. The superior mesenteric vein and portal vein were viable without signs of thrombosis. In the arterial phase there was normal arborization of *truncus coeliacus* and arterial plexus of the liver. Pseudoaneurysm was found in the splenic artery of the size 85 mm × 60 mm which was pulsating (Figure 1).

The patient was presented at a meeting of gastroenterologists, radiologists and surgeons who decided to make surgical intervention.

Intraoperatively, a pulsating formation was found in the region of the body and tail of the pancreas completely filling the space of *bursa omentalis*, forming a hard fibrous structure that was adhered to the transverse colon and transverse mesocolon and the whole back wall of the gizzard. The spleen was dark violet and enlarged of the size about 16 cm × 12 cm × 10 cm with tortuous and rigid splenic artery. The whole pancreas was enlarged and fibrous. Expressed and congested venous collaterals in the gastrosplenic ligament, down to the great curvature of gizzard (vv. *gastricae breves*) were found to the great omentum *(gastroepiploica*) and in retroperitoneum (Figure 2). The gizzard was edematous with hypertrophic front and rear wall.

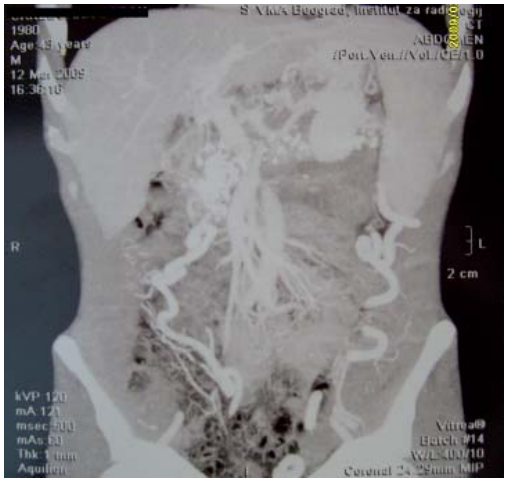


Fig. 1 - Pseudocyst and the enlarged spleen with calcification through the head, body and tail of the pancreas on multislice computed tomography (MSCT) angiography

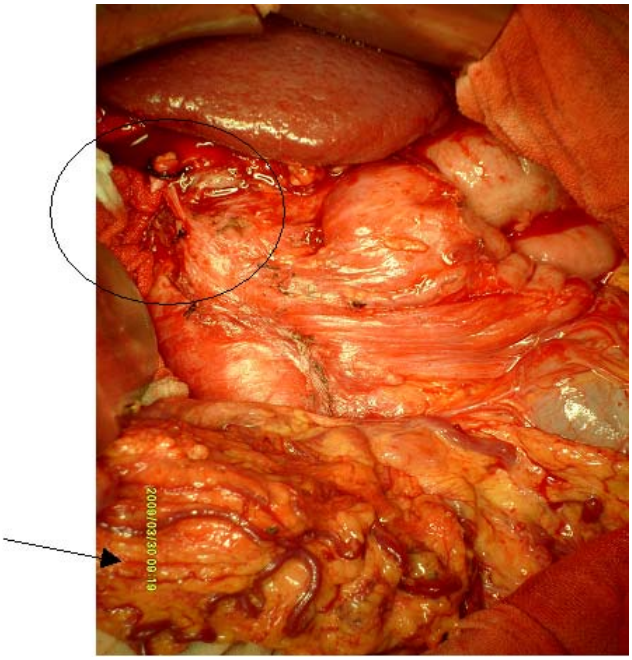


Fig. 2 - Congested venous collaterals, pseudocyst (pseudoaneurysm) and the enlarged spleen

Using transgastrocolic approach we performed meticulous dissection of the gizzard, colon and body and tail of the pancreas, mobilizing the duodenum and the head of the pancreas. Two fine–needle biopsies for *ex tempore* examination were taken from the head and from the junction of the body and tail of the pancreas revealing benign tumor. The splenic artery was double clamped at its beginning and in the splenic hilum (Figure 3). We opened pseudocystic formation at the junction of the body and the tail of the pancreas, which was partly filled with fresh blood, partly with thrombotic masses. After evacuation of the cavity content, the communication

between the lumen of pseudocyst and splenic artery was determined just before branching of the artery. Thrombosis of the splenic vein in the region of pancreatic tail was found. After carefully performed mobilization of the spleen we made splenectomy and distal pancreatectomy with extirpation of pseudoaneurism and proximal ligation of splenic artery. Wirsung's duct was identified and managed with transfixion suture-ligation.

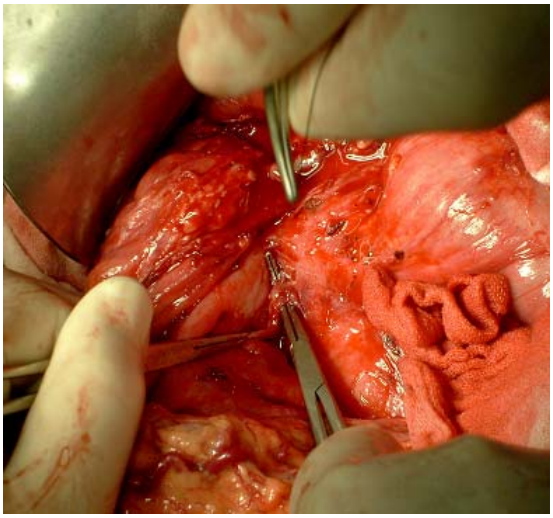


Fig. 3 – Clamping of the splenic artery at each side of pseudoaneurysm

Postoperative course was in order, without bleeding. No pancreatic fistula was registered. The patient was put back to oral food on the 4th postoperative day, and antiagregation therapy was introduced. The patient was discharged on the 13th postoperative day with thrombocytosis of 950 x 10^9/L. One month after discharging thrombocytosis of 1075 x 10^9/L was registered in the patient. The value of amylase in urine and serum was within normal range. US of abdomen registered state after distal splenopancreatectomy without pathological collections in the abdomen. The patient received polyvalent pneumococcal vaccine in dispensary.

Histopathological examination of the extirpated pseudocyst is showed on Figure 4.

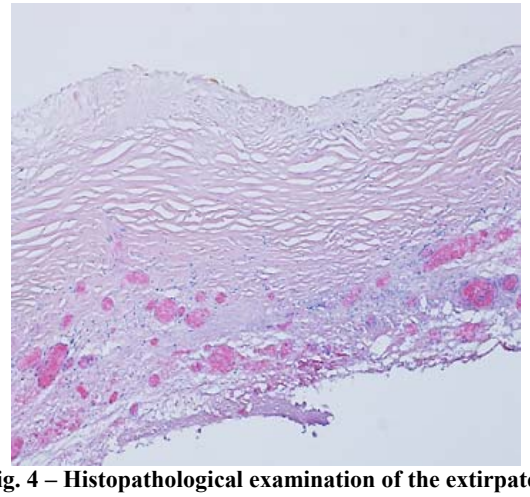


Fig. 4 – Histopathological examination of the extirpated pseudocyst (HE, 5×)

Discussion

Bleeding from pseudoaneurysm based on splenic artery lesions is a potentially fatal complication in patients with chronic pancreatitis. Although rare, it might be caused by trauma [10, 11]. Pseudoaneurysm can be developed with or without existing pseudocystic formations. If it comes to conversion (erosion of pseudocyst into the adjacent court or blood vessels wall) it may cause fatal complications due to rupture and massive hemorrhage (intraperitoneal, retroperitoneal, into the nearby organs and/or ducts) [12]. Bleeding in the bile duct was firstly described by Lower and Farrell [13] in 1931, and named as "*haemosuccus pancreaticus*" by Sanblom [14] in 1970. Pseudocyst with pseudoaneurysm is present in about 10% of patients with chronic pancreatitis [15]. Pseudoaneurysm is caused by enzymatic digestion of blood vessel [16, 17] and/or local compression of the blood vessel wall by pseudocyst [18]. Because of its proximity, the splenic artery is the most frequently affected, about 40% [19]. Further, the incidence of isolated pseudoaneurysms of the splenic artery is low [16].

There are still controversy and disagreement in which cases surgery is needed and what is the gold timing for surgery. Several important factors increase the risk of serious acute hemorrhage: duration of chronic pancreatitis, closeness of pseudocyst to the blood vessels (erosion of pancreatic pseudocyst into a nearby blood vessel is a complication with high mortality rate caused by intraabdominal hemorrhage), communication of pseudocyst with the pancreatic and biliary duct, thrombosis and occlusion of the splenic blood vessels [16, 20].

In 30% of patients the most common clinical manifestation of pseudoaneurysms is abdominal pain [21]. The incidence of intracystic hemorrhage despite the management and diagnosis varies from 6% to 17% [22, 23]. Rupture of pseudoaneurysms was found in 31% of patients with pseudocystic complications of the chronic pancreatitis [24]. According to some authors, the size of pseudoaneurysms from 2 cm to 17 cm is not the cause of their rupture [10, 25]. The frequency of fatal hemorrhage, as complications of chronic pancreatitis, varies from 1.2% to 14.5% (Table 1) [20, 26]. Surgical procedures carry a morbidity and mortality risk of 1.3% - 9% [27, 28].

Endovascular techniques were successfully applied in 75%–85% of patients with morbidity rate of 14%–25%, and mortality rate of 0%–14% [4, 28]. The results of these studies do not recommend percutaneous angioembolisation (PAE) as adequate therapy due to the high percentage of failure, more than 20% [16]. In treatment of the splenic artery pseudoaneurysm surgical or radiological procedures may be used equally [29]. While the effectiveness of embolization is undeniable, it depends on the competence of radiologist. Minimally invasive surgical techniques are used as spare solution for patients with good general condition without other complications of pancreatitis [30]. Previous studies have shown effective and lasting control of bleeding pseudoaneurysm by applying embolization [29, 31], even this procedure has been used as the first treatment with success [32]. Others point out surgery which is always indicated [17, 10, 32]. Distal pancreatectomy and

Table 1

Clasification of hemorrhagic complications in chronic pancreatitis (modified to ref. 12)

(A)	**Pancreatitis–related**
1.	Pseudoaneurysms or arterial wall necrosis and rupture
2.	Gastric-oesophageal varices [splenic vein thrombosis with left– sided portal hypertension (LPH)]
3.	Intracystic hemorrhage from vessels within the pseudocyst wall
4.	Splenic rupture
(B)	**Coexistent pathology**
1.	Peptic ulceration
2.	Gastritis, duodenitis, or stress ulceration
3.	Oesophageal varices (alcoholic liver disease with portal hypertension)
4.	Mallory – Weiss syndrome

splenectomy is the method of choice for complicated hemorrhagic pseudocyst localized in the tail of the pancreas, with very low morbidity and mortality rate [32, 35].

There are several possibilities to solve complicated pseudocysts (greater than 6 cm): 1) percutaneous US application of trombine [3, 36] (when surgery and endovascular embolization are not feasible or not possible), for first-line treatment [36]; 2) endoscopic drainage (very effective in many patients with acceptable complication rate; without possibility to control bleeding, this procedure is contraindicated in hemorrhagic pseudocysts); with endoscopic retrograde cholangiopancreatography (ERCP) is effective in the most cases after embolization [5]; 3) surgery [7–10] (invasive, more traumatic but successful if other procedures fails; there is the possibility to control bleeding [5, 37]); 4) laparoscopic procedures, but only in elective cases [38]; 5) endovascular treatment (transcatheter angioembolisation), can be used as the first line treatment [4, 5, 37].

The high incidence of morbidity and mortality rate requires an essential and active management and multidisciplinary approach to solving complicated pancreatic pseudocysts and complications which accompany it. An optimal approach is determined by the presentation of patients. Surgery and PAE have complementary role. PAE is recommended as the method for hemodynamiclly stable patients. Surgery is reserved for active bleeding, hemodynamiclly unstable patients, severe pain, poor or failed PAE, as well as other complications such as infection and/or external compression of the surrounded organs [16]. In the regional LPH as the result of splenic vein thrombosis is the most common pathology regarding chronic pancreatitis and/or the existence of pseudocyst localized in the tail of the pancreas [34, 35]. The incidence of isolated splenic vein thrombosis at autopsies in patients with the history of chronic pancreatitis was 20%–40% [15, 33]. Splenectomy with distal pancreatectomy is the gold standard for resolving vascular lesions of the pancreas [10]. Also, splenectomy with distal pancreatectomy (treatment in pancreatic pathology) is a curative method for solving regional LPH [32, 34].

Conclusion

Angiography is the most reliable and the safest method for diagnosing hemorrhagic pseudocysts when they clinically present as pseudoaneurysms. Chemoembolization is a method of choice for uncomplicated pseudoaneurysms. In the presented case, a potentially dangerous complication was treated surgically with an excellent postoperative result.

R E F E R E N C E S

1. *Vujic I.* Vascular complications of pancreatitis. Radiol Clin North Am 1989; 27(1): 81–91.
2. *Mallick IH, Winsle MCt.* Vascular complication of pancreatitis. JOP J Pancreas 2004; 5(5): 328–37.
3. *Sparrow P, Asquith J, Chalmers N.* Ultrasonic-guided percutaneous injection of pancreatic pseudoaneurysm with thrombin. Cardiovasc Intervent Radiol 2003; 26(3): 312–5.
4. *Yamaguchi K, Futagawa S, Ochi M, Sakamoto I, Hayashi K.* Pancreatic pseudoaneurysm converted from pseudocyst: transcatheter embolization and serial CT assessment. Radiat Med 2000; 18(2): 147–50.
5. *Udd M, Leppäniemi AK, Bidel S, Keto P, Roth WD, Haapiainen RK.* Treatment of bleeding pseudoaneurysms in patients with chronic pancreatitis. World J Surg 2007; 31(3): 504–10.
6. *Piffaretti G, Tozzi M, Lomazzi C, Rivolta N, Riva F, Caronno R,* et al. Splenic artery aneurysms: postembolization syndrome and surgical complications. Am J Surg 2007; 193(2): 166–70.
7. *El Hamel A, Parc R, Adda G, Bouteloup PY, Huguet C, Malafosse M.* Bleeding pseudocysts and pseudoaneurysms in chronic pancreatitis. Br J Surg 1991; 78(9): 1059–63.
8. *Hsu JT, Yeh CN, Hung CF, Chen HM, Hwang TL, Jan YY,* et al. Management and outcome of bleeding pseudoaneurysm associated with chronic pancreatitis. BMC Gastroenterol 2006; 6: 3.
9. *de Perrot M, Berney T, Bühler L, Delgadillo X, Mentha G, Morel P.* Management of bleeding pseudoaneurysms in patients with pancreatitis. Br J Surg 1999; 86(1): 29–32.
10. *Tessier DJ, Stone WM, Fowl RJ, Abbas MA, Andrews JC, Bower TC,* et al. Clinical features and management of splenic artery pseudoaneurysm : case series and cumulative review of literature. *J Vasc Surg* 2003; 38(5): 969–74.
11. *Sugg SL, Gerndt SJ, Hamilton BJ, Francis IR, Taheri PA, Rodriguez JL.* Pseudoaneurysms of the intraparenchymal splenic artery after blunt abdominal trauma: a complication of nonoperative therapy and its management. J Trauma 1995; 39(3): 593–5.
12. *Ammori BJ, Madan M, Alexander DJ.* Haemorrhagic complications of pancreatitis: presentation, diagnosis and management. Ann R Coll Surg Engl 1998; 80(5): 316–25.
13. *Lower WE, Farrell Jl.* Aneurysm of the splenic artery: report of case and review of the literature. Arch Surg 1931; 23: 182–90.

14. *Sandblom P.* Gastrointestinal hemorrhage through the pancreatic duct. Ann Surg 1970; 171(1): 61–6.
15. *Rignault D, Mine J, Moine D.* Splenoportographic changes in chronic pancreatitis. Surgery 1968; 63(4): 571–5.
16. *Carr JA, Cho JS, Shepard AD, Nypaver TJ, Reddy DJ.* Visceral pseudoaneurysms due to pancreatic pseudocysts: rare but lethal complications of pancreatitis. J Vasc Surg 2000; 32(4): 722–30.
17. *Geokas MC.* The role of elastase in acute pancreatitis. II. Intrapancreatic elastolytic activity in trypsin-induced acute pancreatitis in dogs. Arch Pathol 1968; 86(2): 127–34.
18. *Forsmark CE, Wilcox CM, Grendell JH.* Endoscopy-negative upper gastrointestinal bleeding in a patient with chronic pancreatitis. Gastroenterology 1992; 102(1): 320–9.
19. *Boudghène F, L'Herminé C, Bigot JM.* Arterial complications of pancreatitis: diagnostic and therapeutic aspects in 104 cases. J Vasc Interv Radiol 1993; 4(4): 551–8.
20. *Flati G, Andrén-Sandberg A, La Pinta M, Porowska B, Carboni M.* Potentially fatal bleeding in acute pancreatitis: pathophysiology, prevention, and treatment. Pancreas 2003; 26(1): 8–14.
21. *Agrawal GA, Johnson PT, Fishman EK.* Splenic artery aneurysms and pseudoaneurysms: clinical distinctions and CT appearances. AJR Am J Roentgenol 2007; 188(4): 992–9.
22. *Sankaran S, Walt AJ.* The natural and unnatural history of pancreatic pseudocysts. Br J Surg 1975; 62(1): 37–44.
23. *Kiviluoto T, Kivisaari L, Kivilaakso E, Lempinen M.* Pseudocysts in chronic pancreatitis. Surgical results in 102 consecutive patients. Arch Surg 1989; 124(2): 240–3.
24. *Lankisch PG.* Diagnostic standards in chronic pancreatitis. Exocrine pancreatic function tests. Z Gastroenterol Verh 1991; 26: 58–61. (German)
25. *Abbas MA, Stone WM, Fowl RJ, Gloviczki P, Oldenburg WA, Pairolero PC,* et al. Splenic artery aneurysms: two decades experience at Mayo clinic. Ann Vasc Surg 2002; 16(4): 442–9.
26. *Bittner R.* Surgical management of pancreaticabscess. In: *Beger H, Warshaw AL,Buchler M,* editors. The Pancreas. Oxford, United Kingdom: Blackwell Science; 1998. p. 627–31.
27. *Frey CF.* Hemorrhagic pancreatitis. Am J Surg 1979; 137(5): 616–23.
28. *Guillon R, Garcier JM, Abergel A, Mofid R, Garcia V, Chahid T,* et al. Management of splenic artery aneurysms and false aneurysms with endovascular treatment in 12 patients. Cardiovasc Intervent Radiol 2003; 26(3): 256–60.
29. *Bergert H, Hinterseher I, Kersting S, Leonhardt J, Bloomenthal A, Saeger HD.* Management and outcome of hemorrhage due to arterial pseudoaneurysms in pancreatitis. Surgery 2005; 137(3): 323–8.
30. *Gambiez LP, Ernst OJ, Merlier OA, Porte HL, Chambon JP, Quandalle PA.* Arterial embolization for bleeding pseudocysts complicating chronic pancreatitis. Arch Surg 1997; 132(9): 1016–21.
31. *Beattie GC, Hardman JG, Redhead D, Siriwardena AK.* Evidence for a central role for selective mesenteric angiography in the management of the major vascular complications of pancreatitis. Am J Surg 2003; 185(2): 96–102.
32. *Bresler L, Boissel P, Grosdidier J.* Major hemorrhage from pseudocysts and pseudoaneurysms caused by chronic pancreatitis: surgical therapy. World J Surg 1991; 15(5): 649–52.
33. *Evans GR, Yellin AE, Weaver FA, Stain SC.* Sinistral (left-sided) portal hypertension. Am Surg 1990; 56(12): 758–63.
34. *Sakorafas GH, Sarr MG, Farley DR, Farnell MB.* The significance of sinistral portal hypertension complicating chronic pancreatitis. Am J Surg 2000; 179(2): 129–33.
35. *Bernades P, Baetz A, Lévy P, Belghiti J, Menu Y, Fékété F.* Splenic and portal venous obstruction in chronic pancreatitis. A prospective longitudinal study of a medical-surgical series of 266 patients. Dig Dis Sci 1992; 37(3): 340–6.
36. *McErlean A, Looby S, Lee MJ.* Percutaneous ultrasound-guided thrombin injection as first-line treatment of pancreatic pseudoaneurysm. Cardiovasc Intervent Radiol 2007; 30(3): 526–8.
37. *Bergert H, Dobrowolski F, Caffier S, Bloomenthal A, Hinterseher I, Saeger HD.* Prevalence and treatment of bleeding complications in chronic pancreatitis. Langenbecks Arch Surg 2004; 389(6): 504–10.
38. *Palanivelu C, Senthilkumar K, Madhankumar MV, Rajan PS, Shetty AR, Jani K,* et al. Management of pancreatic pseudocyst in the era of laparoscopic surgery-experience from a tertiary centre. Surg Endosc 2007; 21(12): 2262–7.

Received on July 21, 2009.
Accepted on December 25, 2009.

CASE REPORT

UDC: 618.7-022.::[616.98:579.862
DOI: 10.2298/VSP1107607N

Group A streptococcal cellulitis in the early puerperium

Celulitis izazvan streptokokom grupe A u ranom puerperijumu

Branka Nikolić*, Ana Mitrović*, Svetlana Dragojević-Dikić*, Snežana Rakić*, Zlatica Cakić*, Milena Saranović*, Milan Sikimić†

***University of Belgrade, *School of Medicine,** Obstetrics and Gynecology Clinic "Narodni front", **†Faculty of Sport and Physical Culture, Belgrade, Serbia**

Abstract

Introduction. Infectious diseases caused by *Streptococcus pyogenes*, a member of the group A *Streptococci* (GAS) are among the most common life threatening ones. Patients with GAS infections have a poor survival rate. Cellulitis is a severe invasive GAS infection and the most common clinical presentation of the disease associated with more deaths than it can be seen in other GAS infections. According to the literature data, most cases of GAS toxic shock syndrome are developed in the puerperium. However, there are two main problems with GAS infection in early puerperium and this case report is aimed at reminding on them. The first problem is an absence of awareness that it can be postpartal invasive GAS infection before the microbiology laboratory confirms it, and the second one is that we have little knowledge about GAS infection, in general. **Case report.** A 32-year-old healthy woman, *gravida* 1, *para* 1, was hospitalized three days after vaginal delivery with a 38-hour history of fever, pain in the left leg (under the knee), and head injury after short period of conscious lost. Clinical picture of GAS infection was cellulites. Group A *Streptococcus pyogenes* was isolated in vaginal culture. Rapid antibiotic and supportive treatment stopped development of streptococcal toxic shock syndrome (STSS) and potential multiorganic failure. Signs and symptoms of the infection lasted 25 days, and complete recovery of the patient almost 50 days. **Conclusion.** In all women in childbed with a history of fever early after delivery, vaginal and cervical culture specimens should be taken as soon as possible. Early recognition of GAS infection in early puerperium and prompt initiation of antimicrobial drug and supportive therapy can prevent development of STSS and lethal outcome.

Key words:
streptococcus pyogenes; puerperal infection; cellulitis; shock, septic; diagnosis; drug therapy; treatment outcome.

Apstrakt

Uvod. Infekcije koje izaziva *Streptococcus pyogenes*, predstavnik grupe A streptokoka (GAS), spadaju među one koje mogu da ugroze život. Bolesnici sa razvijenom infekcijom izazvanom GAS imaju nisku stopu preživljavanja. Celulitis je ozbiljna, invazivna GAS infekcija. To je najčešća klinička prezentacija GAS infekcije sa češćim smrtnim ishodom u odnosu na ostale GAS infekcije. Prema podacima iz literature, najveći broj slučajeva toksičnog sindroma u sklopu GAS infekcije razvije se u puerperijumu. Postoje dva problema vezana za GAS infekcije u ovom periodu, pa je i cilj ovog prikaza bio da se na njih, još jednom, podsetimo. Prvi problem vezan je za lekare koji najčešće nisu svesni da se radi o invazivnoj GAS infekciji, pre nego što im mikrobiološka laboratorija to ne potvrdi, dok se drugi odnosi na, generalno, slabije znanje o GAS infekciji. **Prikaz bolesnika.** Bolesnica, stara 32 godine, prvorotka, hospitalizovana je trećeg dana posle porođaja zbog febrilnosti koja se održavala 38 sati, bola u levoj potkolenici i povrede na glavi koju je zadobila u toku kratkotrajnog gubitka svesti. Klinička slika GAS infekcije bio je celulitis. *Streptococcus pyogenes* grupe A izolovan je u kulturi vaginalnog brisa. Brzo uključivanje antibiotika i suportivne terapije sprečilo je razvoj streptokoknog toksičnog šok sindroma (STSS), kao i potencijalnu multiorgansku disfunkciju. Znaci i simptomi infekcije bili su prisutni 25 dana, dok je potpuni oporavak bolesnice trajao gotovo 50 dana. **Zaključak.** Kod svih porodilja koje su febrilne nakon porođaja, trebalo bi što je pre moguće uraditi kulturu iz vaginalnog i cervikalnog brisa. Rano prepoznavanje GAS infekcije i brzo započinjanje antimikrobne i suportivne terapije može da spreči razvoj STSS i, posledično, smrtni ishod.

Ključne reči:
streptococcus pyogenes; infekcija, puerperijum; celulitis; šok, septički; dijagnoza; lečenje lekovima; lečenje, ishod.

Introduction

Group A *Streptococci* (GAS) cause infections of public health meaning. Clinical picture varies from local infections such as pharyngitis or vaginitis to severe invasive GAS cellulitis, meningitis, myositis and, even, multiorganic failure as streptococcal toxic shock syndrome (STSS) or necrotysing fasciitis (NF) [1–4]. Infectious diseases caused

Correspondence to: Branka Nikolić, Požeška 14, 11 000 Belgrade, Serbia. Phone: +381 11 206 83 45. E-mail: bolnicanf@sbb.rs, n_branka@eunet.rs

by the group A *Streptococcus pyogenes*, known as "flesh eating bacteria", are among the most common life threatening ones. The presence of bacterial egzotoxyns in blood can cause generalized intoxication and shock syndrome with cytotoxic effect and fatal outcome within 48–96 hours. Bacteriemia occurs in only 0.3% of febrile patients [1, 2, 4]. Besides fever, hypothermia, hypotension, tachycardia, hypoalbuminemia, oliguria can be observed and have to be carefully monitored [1–3, 5–8].

Literature data suggest that letal outcome is possible within 30 days from the confirmation of *Streptococcus pyogenes* presence in any culture specimen. Good prognosis of GAS infection depends of early recognition and intensive antimicrobial and supportive treatment which must last until signs of the infection are completely retired. Extremities, trunk and pelvis are most often GAS infected. Cellulitis, an invasive GAS infection, is the most common clinical presentation of the disease. Compromising subcutaneous tissue, fascia and adipose tissue it is associated with serious sequels and more deaths (30%) than other GAS infections [1–3, 9–11]. Thirty-day survival after a positive culture specimen has been confirmed is significantly reduced among patients in whom the developed STSS and 26% of these patients die with a day of specimen collection [1, 2, 8].

Severe pain may be the only early symptom of streptococcal myositis and cellulitis. According to the literature most STSS develop in puerperium within 42 days [1, 2, 6].

There are two problems with GAS infection, and this case report is aimed at reminding on them. The first problem is the absence of awareness that it can be postpartal invasive GAS infection before the microbiology laboratory confirms it. The second one is that we have little knowledge about GAS disease, in general [4, 9].

Case report

A 32-year old woman, *gravida* 1, *para* 1, hospitalised on the day 3 after delivery with a 38-hour history of fevers (38–40.3ºC), and pain in the left leg under the knee. There were no edema, redness or visible skin abrasions or minor local trauma on the left leg. Also, the patient had a head injury in occipital region that she did not mentioned because she hardly remembered what had happened during a period of mental changes when she had felt down. Head injury was discovered during the external examination (3 sutures placed). Her pregnancy and labour were uneventful prior to these symptoms.

On the hospital day 1 her blood pressure and pulse were 100/65 mmHg and 130 beats/minute, respectively. Laboratory values included white blood cell count of 20.3×10^9/L, hematocrit of 33%, platelet count of 215×10^9/L, and C reactive protein (CRP) of 459 mg/L (referent value 0–5 mg/L). Hypoalbuminemia was also associated with the mentioned laboratory values. Blood, vaginal and cervical culture, urinalysis, and urine culture were performed before the antibiotic treatment. This is a part of standard procedure in patients in puerperium with more than 24 hours of faver history.

Antibiotic (ceftriaxon 2 × 2 g intravenously), albumin, and electrolyte solutions containing salts were administrated. During the first 6 days 6 liters of electrolyte solutions were given. There were no changes in systolic blood pressure. Renal function was not compromised.

On the hospital day 2, body temperature of the patient was 38–39.2ºC. The patient had more intensive pain along the whole left leg followed with edema and redness. Because of intensive pain in the left leg with limited mobility the patient was examined by vascular surgeon. Thrombophlebitis and deep venous thrombosis were excluded. Unilateral cellulitis cruris was confirmed. The skin was without abrasions. There were no clinical or laboratory data for STSS (not for organ damage, including renal failure, adult respiratory distress syndrome, disseminated intravascular coagulation and serious mental changes). Blood pressure was 110/65 mmHg, white blood cell count had decreasing rate (14.0×10^9/L), while CRP was increased (463.7 mg/L).

On the hospital days 3–4, two blood cultures were negative for GAS. Also, skin, throat, cervical and urine cultures were negative for GAS. Vaginal culture was positive for the group A *Spreptococcus pyogenes*, sensitive to ceftriaxone and clindamycin.

As above mentioned, ceftriaxon was administrated (2 × 2 g, intravenously) on the hospital day 1, then continued in a dose of 2 g, daily. Clindamycin (3 × 300 mg, intravenously) was added to the therapy on the hospital day 3. Blood pressure was 115/70 mmHg, white blood cell count and CRP had decreasing rate (12.4×10^9/L, and 382.9 mg/L, respectively). Throat and chest exams were done and no pathological changes were confirmed. Repeated exam of the skin culture remained negative.

After 6 days of the antibiotic treatment redness, edema and pain were localized under the knee with a limited mobility of the left leg. These changes had slow regression during next 20 hospital days. White blood cell count showed decreasing rate to 7.7×10^9/L, as well as CRP which started to decrease significantly after the hospital day 11 (109.3 mg/L). Repeated exams of vaginal and cervical cultures remained negative.

On the hospital day 9 localised painful redness under the left knee stayed unchanged (Figure 1). Blood pressure increased to 165/95 mmHg. Antihipertensive therapy was administrated during the next two weeks and blood pressure was normalised. The patient had problem with high pressure during the last trimester of pregnancy. Hypotension during the first days of hospitalization was one of the early symptoms for invasive streptococcal infection. Body temperature remained at 37.4–37.8ºC in spite of antibiotic treatment given according to the antibiogram.

From the hospital day 10 meropenem (3 × 0,5 g, intravenously) and vancomycin (2 × 1 g, intravenously) were administrated instead of ceftriaxone and clindamycine. Also, vancomycin was administrated orally (125 mg, daily) to prevent pseudomembranous enterocollitis. Two days after, her body temperature was 37.2ºC. The patient was subfebrile until the 25th day of hospitalization when the patient became afebrile.

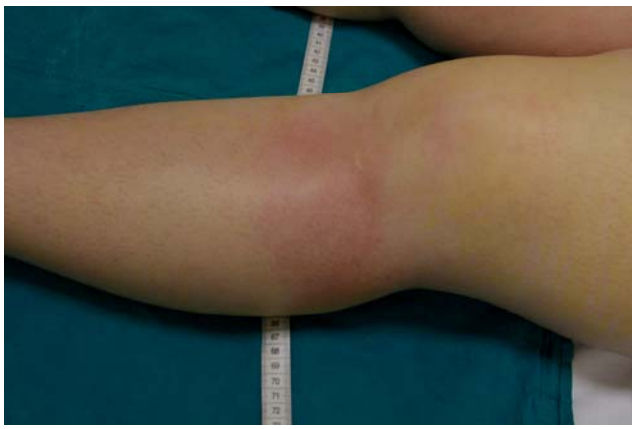


Fig. 1 – Localized painful redness under the left knee on the hospital day 9

Pain and redness withdrawed on the hospital day 26. Edema lasted 10 days more (Figure 2). Walking was normalized after edema withdrowal (Figure 3) and under the professional controlled exercises which lasted two weeks. From the beginning of the disease to the complete recovery of the patient, the treatment lasted almost 50 days.

Fig. 2 – Edema of the left leg 30 days after GAS infection beginning

Fig. 3 – Normal appearance of the left leg in the patient after edema withdrowal

Discussion

The most common initial symptoms of streptococcal infection are pain and fever, followed with nausea, vomiting and diarrhea. Severe pain may be the only early symptom for streptococcal myositis and cellulitis. Bacteriemia occurs in only 0.3% of febrile patients. GAS bacteremia is rare event among persons 14–40 years old, but puerperal sepsis account for most bacteremia in this age group. Infection usually involves one extremity. Twenty percent of patients have an influenza-like syndrome. Confusion is present in 55% of patients, and some of them can in to a coma. Necrotizing fasciitis or myositis is present in 70% of patients, and a diffuse scarlatine-like erythema in 10% of patients [1–3].

The nature of *Streptococcus pyogenes* infection can be dramatic and severe. The most common early clinical signs of soft tissue infection in puerperium are fever, strong pain or/and localized erythema. Also hypotension, hypotermia, hypoalbuminemia, hypocalcemia, mild leucocytosis, increased serum creatinin, need serious diagnostic and treatment approach. Specimens have to be collected from the vagina (in puerperium), nose, throat, and skin. Urinalysis, urinoculture, and hemoculture, also have to be done. Streptococcal infections and sequels treatment and recovery can last more than 4 weeks [1–4].

The presented patient had the history of 38-hour fever, pain, hypotension, hypoalbuminemia and conscious lost with head injury in a short period before the hospitalization. Also, there was no visible tissue trauma before cellulitis developed. The full recovery of the patient lasted 50 days.

Puerperal GAS infection can appear as cellulitis within 42 days after delivery. According to the reported results in literature, cellulitis, the most common GAS infection, has been associated with more deaths (30% patients) compared with other forms of the disease. Survival during 30 days after positive culture confirmation has been significantly reduced among patients in whom STSS developed, and approximately 28% of these patients have died within a day of culture specimen collection [1–6].

Numerous GAS infection cases develop within 24 to 72 hours after minor nonpenetrating trauma prograding to severe septic shock syndrome with hypothermia, hypotension, multiorganic failure, disseminated intravascular coagulation and death [9–11]. Rapid antibiotic and supportive treatment has a great impact on GAS infection outcome. A number of studies show that penicillins and cephalosporins have proven efficacy in treating of GAS infections. According to investigations showing penicillins failure in the presence of numerous streptoccoci and that serious GAS infections treated with penicillins respond less well to them and are associated with high mortality and morbidity rates, cephalosporins and clindamycin have become antibiotics of choice. Cephalosporins have proven efficacy in cellulitis treatment caused by *Streptococcus pyogenes* [2, 4–6]. Our results in preveting STSS development in this case confirm it. Beta-lactam antibiotics continue to be very effective for GAS infections because of a prolonged effect after treatment and very rare resistancy [2].

However, prolonged treatment of sequels may need the use of other antimicrobial drugs as we done in this case administrating meropenem and vancomycin.

Conclusion

In all women in childbed with a history of fever early after delivery, vaginal and cervical culture specimens should be taken as soon as possible. Early recognition of group A *Streptococcus pyogenes* infection or GAS invasive disease according to microbiology confirmation and prompt initiation of antibiotic and supportive therapy are very important in prevention of developing STSS and fatal outcome in these patients.

Acknowledgement

We thank the personnel of the Microbiology Laboratory, Obstetrics and Gynecology Clinic "Narodni front", Belgrade, for their professional assistance in performing microbiologic analyses.

R E F E R E N C E S

1. *Lamagni TL, Neal S, Keshishian C, Alhaddad N, George R, Duckworth G,* et al. Streptococcus pyogenes infections, United Kingdom, 2003-2004. Emerg Infect Dis 2008; 4(2): 202–9.
2. *Stevens DL.* Streptococcal toxic-shock syndrome: spectrum of disease, pathogenesis, and new concepts in treatment. Emerg Infect Dis 1995; 1(3): 69–78.
3. *Lamagni TL, Darenberg J, Luca-Harari B, Siljander T, Efstratiou A, Henriques-Normark B,* et al. Epidemiology of severe Streptococcus pyogenes disease in Europe. J Clin Microbiol 2008; 46(7): 2359–67.
4. *Rainbow J, Jewell B, Danila RN, Boxrud D, Beall B, Van Beneden C,* et al. Invasive group a streptococcal disease in nursing homes, Minnesota, 1995-2006. Emerg Infect Dis 2008; 14(5): 772–7.
5. *O'Loughlin RE, Roberson A, Cieslak PR, Lynfield R, Gershman K, Craig A,* et al. The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000-2004. Clin Infect Dis 2007; 45(7): 853–62.
6. *Neal S, Beall B, Ekelund K, Henriques-Normark B, Jasir A, Johnson D,* et al. International quality assurance study for characterization of Streptococcus pyogenes. J Clin Microbiol 2007; 45(4): 1175–9.
7. *Lamagni TL, Neal S, Keshishian C, Powell D, Potz N, Pebody R,* et al. Predictors of death after severe Streptococcus pyogenes infection. Emerg Infect Dis 2009; 15(8): 1304–7.
8. *Centers for Disease Control and Prevention.* Active bacterial core surveillance report. Emerging Infections Program Network, Group A Streptococcus, 2004. Atlanta: The Centers; 2005 [cited 2007 Jun 4]. Available from: http://www.cdc.gov/ncidod/dbmd/abcs/survreports/gas04.pdf
9. *Lamagni TL, Efstratiou A, Vuopio-Varkila J, Jasir A, Schalén C.* The epidemiology of severe Streptococcus pyogenes associated disease in Europe. Euro Surveill 2005; 10(9): 179–84.
10. *World Health Organization.* Programme on streptococcal diseases complex. Report of a consultation; 1998 Feb 16–19; Geneva. EMC/BAC/98.7. Geneva: WHO; 1998.
11. *Schalén C.* European surveillance of severe group A streptococcal disease. Euro Surveill 2002; [cited 2007 Jun 4]. Available from http://www.eurosurveillance.org/ew/2002/020829.asp

Received on April 22, 2010.
Revised on July 8, 2010.
Accepted on July 20, 2010.

P R I K A Z S L U Č A J A

UDC: 616.132.2::616.12-009.72-06-08
DOI: 10.2298/VSP1107611P

Vazospastična angina pektoris komplikovana akutnim infarktom miokarda i kompletnim atrioventrikularnim blokom

Vasospastic angina pectoris complicated by acute myocardial infarction and complete atrioventricular block

Milan Pavlović, Goran Koraćević, Snežana Ćirić Zdravković, Nebojša Krstić, Aleksandar Stojković, Miodrag Damjanović, Danijela Djordjević

Klinički centar Niš, Klinika za kardiovaskularne bolesti, **Niš, Srbija**

Apstrakt

Uvod. Prolongiran vazospazam sa produženim prekidom protoka u koronarnoj arteriji može dovesti do pojave nekroze miokarda sa porastom kardiospecifičnih enzima, a može biti praćen i poremećajima srčanog ritma, sinkopom, čak i naglom smrću. **Prikaz bolesnika.** Bolesnik, star 55 godina, prilikom izlaganja hladnoći osetio je stežući bol iza grudne kosti, u trajanju od 20-ak minuta tokom koga je došlo do gubitka svesti. Na elektrokardiogramu (EKG) nađen je kompletan atrioventrikularni (AV) blok i izražena elelvacija ST segmenta u inferiornim odvodima. Ubrzo je došlo do povlačenja EKG promena i normalizovanja elektrokardiograma, ali sa porastom aktivnosti kardiospecifičnih enzima u serumu. Koronografija je pokazala normalan nalaz leve koronarne arterije i suženje na dužem segmentu medijalnog dela desne koronarne arterije, koje se izgubilo posle intrakoronarnog davanja nitroglicerina. Određena je peroralna terapija diltiazemom, amlodipinom, isosorbid mononitratom, molsidominom, aspirinom, simvastatinom. Nakon sedam meseci terapija je obustavljena, ali je došlo do ponavljanja anginoznih napada i zabeležena je tranzitorna elevacija ST segmenta u inferiornim odvodima u EKG-u, uz pojavu učestalih ventrikularnih ekstrasistola, bez porasta enzima. Ponovno uvođenje terapije dovelo je do povlačenja tegoba. **Zaključak.** Koronarni dilatatori u maksimalnim dozama mogu prevenirati napade vazospastične angine.

Ključne reči:
angina pektoris, varijanta; vazokonstrikcija; infarkt miokarda; srce, blok; lečenje lekovima.

Abstract

Background. A prolonged coronary artery spasm with interruption of coronary blood flow can lead to myocardial necrosis and increase of cardiospecific enzymes and can be complicated with cardiac rhythm disturbances, syncopc, or even sudden cardiac death. **Case report.** A 55-year old male felt a severe retrosternal pain when exposing himself to cold weather. The pain lasted for 20 minutes and was followed by the loss of conscience. Electrocardiogram (ECG) showed a complete antrioventricular (AV) block with nodal rhythm and marked elevation of ST segment in inferior leads. Electrocardiogram was soon normalized, but serum activities of cardiospecific enzymes were increased. Coronarography showed normal findings for the left coronary artery and a narrowing at the middle part of the right coronary artery, which disappeared after intracoronary application of nitroglycerine. The following therapy was prescribed: Diltiazem, Amlodipin, Isosorbid mononitrate, Molisdomin, Atrovastatin, Aspirin and Nitroglycerine spray. After 7 months medicaments were abandoned and the patient experienced again reccurent chest pain episodes at rest. Transitory ST segment elevation was recorded in inferior leads of ECG, but without increase of cardiospecific enzymes serum activities. After restoration of the medicament therapy anginal episodes ceased. **Conclusion.** Coronary dilators in maximal doses can prevent attacks of vasospastic angina.

Key words:
angina pectoris, variant; vasoconstriction; myocardial infarction; heart block; drug therapy.

Uvod

Za razliku od klasične angine pektoris koja se ispoljava u toku fizičkog napora, vazospastična angina pektoris pojavljuje se u miru, ponavlja u određeno doba dana, najčešće u ranim jutarnjim časovima. Ovaj sindrom prvi je opisao Prinzmetal 1959. godine i pretpostavio da se u osnovi poremećaja nalazi prolazni spazam koronarne arterije [1]. Izražen i prolongiran vazospazam sa produženim prekidom protoka u koronarnoj arteriji može dovesti do pojave nekroze miokarda sa porastom aktivnosti kardiospecifičnih enzima u serumu. Epizode koronarnog spazma mogu biti praćene poremećaji-

Correspondence to: Milan Pavlović, Klinički centar Niš, Klinika za kardiovaskularn bolesti, Branka Miljkovića 51, 18 000 Niš, Srbija. Tel.: +381 18 530 472. E-mail: milanpa@eunet.rs

ma srčanog ritma i provođenja, može doći i do pojave sinkopa, čak i nagle srčane smrti.

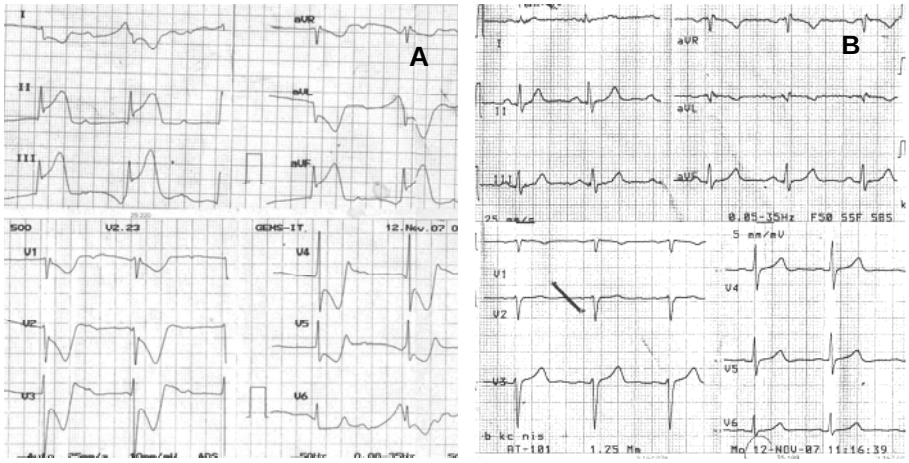
Prikaz bolesnika

Bolesnik, star 55 godina, prilikom izlaganja hladnoći posle izlaska iz tople prostorije, osetio je stežući bol iza grudne kosti. Bol je bio rastućeg intenzieta, trajao je 20-ak minuta, bio je praćen gušenjem i malaksalošću, a zatim je došlo do gubitka svesti i pada na ulici. Bolesnik je bio ranije lečen od opstruktivne plućne bolesti. Pušio je preko 20 cigareta dnevno, i nije imao druge faktore rizika za nastanak koronarne bolesti.

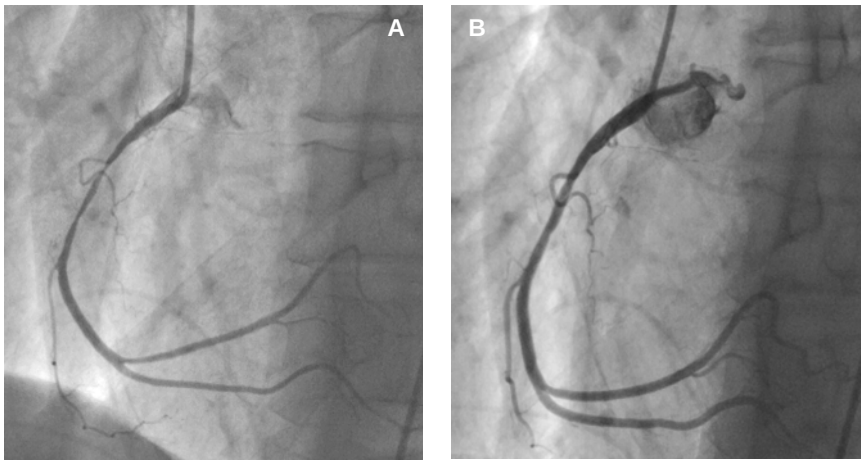
Pri pregledu bio je bled, hladno preznojen. Prilikom ukazivanja prve pomoći izmeren mu je snižen krvni pritisak 90/60 mmHg. Nađena je bradikardija i srčani tonovi oslabljenog inteziteta. Na elektrokardiogramu (EKG) pri prijemu bolesnika, posle intravenskog davanja fiziološkog rastvora i ampule atropina (1 mg) na terenu od strane lekara hitne pomoći, nađen je kompletan AV blok, sa nodalnim ritmom i srčanom frekvencijom od 50/min. Registrovana je elevacija ST segmenta od 8 mm u inferiornim odvodima D3, AVF, elevacija ST manjeg stepena u D2, i slika depresije ST segmenta u recipročnim odvodima D1 i AVL. Ubrzo je došlo do popravljanja elektrokardiograma, nestanka kompletnog AV bloka i pojave sinusnog ritma sa srčanom frekvencijom 80/min (slika 1).

Bolesnik je hospitalizovan i ehokardiografski utvrđena je normalna veličina leve komore. Registrovana je hipokinezija inferoposteriornog zida u bazalnom delu i očuvana globalna kontraktilna funkcija leve komore. Na radiografiji (Rtg) pluća i srca nađena je normalna srčana senka, hiperinflacija pluća i spuštene plućne baze. Na 24-časovnom Holter elektrokardiogramu registrovan je sinusni ritam sa minimalnom srčanom frekvencijom od 58/min, maksimalnom frekvencijom od 119/min i srednjom srčanom frekvencijom od 72/min. Nije bilo pauza u srčanom radu (> 2 sec), niti poremećaja u atrioventrikularnom provođenju. Zabeležene su retke atrijalne i ventrikularne ekstrasistole i nije bilo značajnijih promena ST segmenta niti T-talasa. Laboratorijske analize pokazale su povećane vrednosti troponina I 0,36 μg/L i CKMB 10,4 ng/mL, (gornja granica normalnih vrednosti je 0,04 μg/L za troponin i 3,5 ng/mL za CKMB) i normalne vrednosti glikemije, lipida, ureje, kreatinina, elektrolita i krvne slike.

Koronarografija je pokazala normalni nalaz sistema leve koronarne arterije i suženje na dužem segmentu medijalnog dela desne koronarne arterije (slika 2). Posle intrakoronarnog davanja nitroglicerina došlo je do izraženog povlačenja suženja desne koronarne arterije. Rezidualna stenoza medijalnog segmenta posle vazodilatacije nije više značajno sužavala lumen desne koronarne arterije, ali je mogla da ukaže na bolest zida arterije kao teren za nastanak vazospazma. Predloženo je dalje medikamentno lečenje.



Sl. 1 – Elektrokardiogram u toku epizode vazospastične angine pektoris (A) i tri sata posle ataka (B)



Sl. 2 – Arteriogram desne koronarne arterije pre (A) i posle (B) intrakoronarnog davanja nitroglicerina

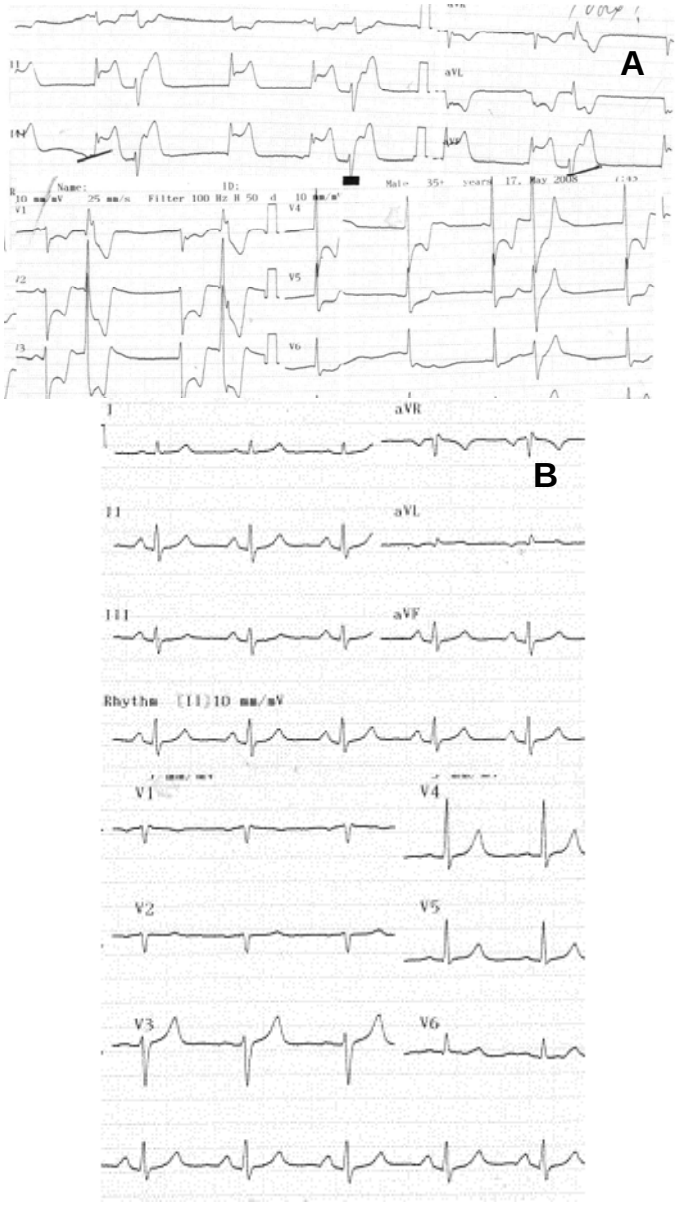
Bolesnik je primljen sa slikom akutnog infarkta inferiornog zida, sa porastom aktivnosti kardiospecifičnih enzima u serumu. Infarkt je u akutnoj fazi komplikovan pojavom Adam Stokes-ovog sindroma, u sklopu tranzitornog kompletnog AV bloka. Koronarografijom je nađeno postojanje spazma desne koronarne arterije i odsustvo fiksne opstruktivne koronarne stenoze.

Insistirano je da bolesnik prestane da puši i predloženo dobro utopljavanje prilikom izlaganja hladnoći. Data je sledeća peroralna medikamentna terapija: diltiazem 90 mg, ujutru i uveče; amlodipin 5 mg uveče; isosorbid mononitrat 40 mg, ujutru i u podne; molsidomin 8 mg uveče; aspirin 100 mg ujutru; simvastatin 40 mg uveče i nitroglicerin u spreju po potrebi. Predloženo je da bolesnik nastavi raniju terapiju za opstruktivnu bolest pluća i da ponovi 24-časovni Holter EKG i test opterećenja. U toku hospitalnog lečenja bolesnik nije imao angionoznih tegoba niti ishemijskih promena na EKG-u.

Nakon sedam meseci bolesnik je postepeno smanjivao doze medikamentne terapije. Iz terapije su isključeni diltiazem, amlodipin i isosorbid mononitrat. Ponavljano su počeli da se javljaju bolovi u sredogruđu u trajanju od desetak minuta, u miru, izraženije u ranim jutarnjim časovima, zbog čega je bolesnik ponovo hospitalizovan.

Na EKG-u u toku anginoznog napada u miru (slika 3) ponovo je zabeležena tranzitorna elevacija ST segmenta u D2, D3 i AVF, i depresija ST segmenta u D1, AVL, kao i u prekordijalnim odvodima V1–V5, uz pojavu učestalih ventrikularnih ekstrasistola. Neposredno posle prestanka epizode vazospastične angine došlo je do potpune normalizacije EKG-a. Troponin i drugi markeri nekroze miokarda prilikom ove hospitalizacije nisu bili povećani i nisu registrovani poremećaji atrioventrikularnog provođenja.

Bolesniku je vraćena intenzivna medikamentna terapija i u toku narednih meseci nisu se više javljale anginozne tegobe. Na Holter EKG-u nije registrovana ishemija miokarda.



Sl. 3 – Elektrokardiogram nakon sedam meseci, a posle obustavljanja terapije: u toku epizode vazospastične angine pektoris (A) i 30 minuta posle ataka (B)

Diskusija

Za razliku od klasične angine pektoris koja se ispoljava u toku fizičkog napora, vazospastičnu anginu pektoris ne provocira fizičko opterećenje, pojavljuje se u miru, ponavljano u određeno doba dana, najčešće u ranim jutarnjim časovima. Ovaj sindrom prvi je opisao Prinzmetal 1959. godine i pretpostavio da se u osnovi poremećaja nalazi prolazni spazam koronarne arterije [1]. Ova koncepcija kasnije je potvrđena koronarnom arteriografijom i testovima koji provociraju vazospazam [2, 3]. Najčešće se spazam pojavljuje na jednom segmentu koronarne arterije, može dovesti do potpunog prekida protoka krvi i pojave transmuralne ishemije miokarda, sa anginoznim bolom i elevacijom ST segmenta na elektrokardiogramu.

Spazam koronarne arterije može nastati zbog disfunkcije endotela arterije i predominacije vazokonstriktornih uticaja nad vazodiltatornim faktorima. Smanjena lokalna produkcija vazodilatatornih supstanci – azot oksida i prostaciklina i/ili povećano oslobađanje vazokonstriktornih supstanci – kateholamina angiotenzina II, tromboksana A2, serotonina, endotelina, histamina, leukotriena i vazopresina, mogu dovesti do konstrikcije glatke muskulature medije arterije i pojave spazma koronarne arterije. Poremećaj simpatičke inervacije i lokalni disbalans simpatičke i parasimpatičke aktivnosti na nivou koronarnih arterija, može imati za posledicu povećanje senzitivnosti vazokonstriktornih alfa adrenergičkih receptora i pojavu koronarnog spazma [1].

Spazam koronarne arterije može nastati na terenu ateroma koji značajno sužava lumen arterije, ali i na segmentu arterije bez vidljivog fiksnog aterosklerotskog suženja. Produženi spazam koronarne arterije sa prekidom protoka krvi može dovesti i do aktivacije i agregacije trombocita, kao i pokretanja kaskade koagulacije, sa razvojem koronarne tromboze i infarkta miokarda [1].

Koronarni spazam može biti superponiran i na ateromu sa rupturom ili erozijom endotela i imati manji ili veći značaj u evoluciji akutnog koronarnog sindroma. Aktivacija trombocita na mestu rupturisanog trombotičkog plaka dovodi do oslobađanja tromboksana A2 i serotonina koji imaju vazokonstriktorni uticaj i dovode do daljeg sužavanja lumena koronarne arterije [1].

Epizode spazma koronarne arterije u vazospastičnoj angini pektoris, po pravilu, javljuju se nevezano od fizičkog napora, u miru, pa je termin varijantna angina i uveden da označi bol u sredogrudju sa drugačijim karakteristikama pojave u odnosu na klasičnu anginu pektoris. U varijantnoj angini bol se pojavljuje u cikličnim intervalima i ponavlja se obično u isto doba dana, češće u ranim jutarnjim časovima. Periodi pogoršanja tegoba mogu biti kombinovani sa dužim ili kraćim intervalima smanjenja tegoba i stabilizovanja stanja, u trajanju od nekoliko nedelja ili meseci. Studije sa 24 časovnim ambulatornim EKG-om bolesnika sa vazospastičnom anginom pektoris pokazale su da postoje i asimptomatske ishemijske epizode, izraženije u ranim jutranjim časovima. Nađeno je i postojanje udruženosti koronarnog spazma i drugih vazospastičnih poremećaja kao što su migrena, Raynaud fenomen itd. Bolesnici sa varijntnom anginom pektoris su mlađi u odnosu na bolesnike sa klasičnom anginom pektoris. Kod njih su obično u manjoj meri prisutni klasični faktori rizika od ateroskleroze, sa izuzetkom pušenja [1].

Izražen i prolongiran vazospazam sa produženim prekidom protoka u koronarnoj arteriji može dovesti do pojave nekroze miokarda sa porastom aktivnosti kardiospecifičnih enzima u serumu i do razvoja *non* Q ili Q-infarkta miokarda [4–7]. Može doći i do pojave tranzitornog Q-zupca u elektrokardiogramu, što je posledica prolaznog gubitika električne aktivnosti membrane miokardnih ćelija u toku spazma. Napad vazospastične angine pektoris može, u rećim situacijama provocirati pojavu akutne srčane insuficijencije [1].

Epizode koronarnog spazma mogu biti praćene poremećajima srčanog ritma i provođenja, može doći i do pojave sinkopa, čak i nagle srčane smrti [5, 6]. Pojava transmuralne ishemije miokarda uzrokovane spazmom koronarne arterije može dovesti do poremećaja srčanog ritma koji se mogu ispoljiti simultano sa nastankom ishemije, a mogu se pojaviti i kasnije, u toku reperfuzije, sa popuštanjem koronarnog spazma [7]. U toku spazma koronarne arterije može doći do prolaznih poremećaja AV ili intraventrikularnog provođenja i bradikardije. Pojava kompletnog AV bloka može biti komplikovana nastankom ventrikularnih malignih poremećaja ritma. Kod bolesnika sa varijantnom anginom učestalost pojave sinkope iznosi i do 25% i predstavlja znak nepovoljnije prognoze bolesnika.

Ključ dijagnoze vazospastične angine je pojava tranzitorne elevacije ST segmenta, udružene sa anginoznim bolom u mirovanju. Ambulantni 24 časovni Holter EKG pokazao je da preko 70% ishemijskih epizoda u varijantnoj angini pektoris nije praćeno simptomima. U provociranju vazospazma koristi se test hiperventilacije i test izlaganja hladnoći. Hiperventilacija u toku 6 min, u jutarnjim časovima, izolovana ili udružena sa fizičkim naporom u testu opterećenja, je metod za ispitivanje varijantne angine pektoris. Ovaj test nema senzitivnost kakvu imaju testovi sa ergonovinom ili acetilholinom [2, 3]. Bolesnici sa pozitivnim nalazom u testu imaju izraženiju aktivnost bolesti i ozbiljnije poremećaje srčanog ritma i provođenja, a mogu imati i spazam na više segmenata različitih koronarnih arterija.

U lečenju varijantne angine pektoris neophodno je prekinuti pušenje. Najvažniji lekovi su antagonisti kalcijuma u kombinaciji sa dugodelujućim nitratima. Potrebno je da se medikamentna terapija sprovodi u dužem vremenskom periodu.

Antagonisti kalcijuma smanjuju koncentraciju intracelularnog kalcijuma, redukuju kontraktilnost glatkih mišićnih ćelija u medijalnom sloju arterije i smanjuju tonus koronarnih arterija. Efikasni su u prevenciji koronarnog spazma i treba ih prepisivati u maksimalno podnošljivoj dozi [8]. Ukoliko se jednim antagonistom kaclijuma ne postigne zavodoljavajući rezultat, može se dodati antagonist kalcijuma druge klase. Mogu se kombinovati diltiazem ili verapamil sa nekim dugodelujućim dihidropiridinskim preparatom i, na ovaj način, dobija se dobar i balansiran efekt na srčanu frekvenciju. Kod napada vazospastične angine pektoris može se koristiti, pored nitroglicerina, i nifedipin sublingvalno u cilju popuštanja koronarnog spazma, uz rizik pojave hipotenzije i reflek-

sne tahikardije. Antagonisti kalcijuma druge generacije, amlodipin i felodipin, i treće generacije, lercanidipin, takođe, pokazali su efikasnost u prevenciji pojave vazospazma, a pogodnostima je u doziranju jednom dnevno, zbog produženog trajanja dejstva [8, 9].

U terapiji vazospastične angine pektoris važno mesto imaju i nitrati. Nitroglicerin sublingvalno ili intravenski obično brzo prekida napad vazospastične angine, a dugodelujući nitrati korisni su u prevenciji anginoznih napada. Nitrati su značajni zbog koronarne vazodilatacije, a takođe i zbog smanjenja miokardne potrošnje kiseonika. Glavno ograničenje nitrata je tolerancija, koja se brzo razvija sa kontinuiranom primenom leka. Ovaj problem može se prevazići uvođenjem pauza uzimanja leka u toku noći, tako što se nitrat dozira ujutru i u podne, uz pokrivanje noći molsidominom, a ne nitratnim koronarnim dilatatorom [8].

ACE inhibitori pokazali su nešto nižu efikasnost u prevenciji koronarnog spazma, u poređenju sa antagonistima kalcijuma i nitratima. Ukoliko i pored pune terapije maksimalnim dozama dva antagonista kalcijuma i nitrata i dalje postoje napadi vazospastične angine pektoris, može se pokušati sa uvođenjem alfa blokatora doksazosina [8].

Magnezijum ispoljava neke efekte antagonista kalcijuma i doprinosi stabilizaciji bolesti. Kardioselektivni beta blokatori mogu se koristiti kod ovih bolesnika, ali samo zajedno sa nitratima i dihidropiridinskim antagonistima kalcijuma. Neselektivni beta blokatori se ne preporučuju, s obzirom da blokadom beta-2 receptora koronarnih arterija mogu dovesti do predominacije vazokonstriktornog uticaja preko slobodnih alfa receptora i pogoršanja napada vazospazma. Aspirin u maloj dozi indikovan je kod bolesnika sa varijantnom anginom, a posebno značajno mesto ima u akutnom koronarnom sindromu. Statini popravljaju funkciju endotela koronarne arterije i doprinose stabilizaciji vazospastične angine pektoris [10].

Kada dođe do remisije simptoma može se pokušati postepeno smanjenje medikamentne terapije, ali je ipak potrebno ostaviti dugodelujući antagonist kalcijuma. Bolesnici sa vazospastičnom anginom komplikovanom tranzitornim kompletnim AV blokom ili malignim srčanim tahiaritmijama imaju povećan rizik od nagle srčane smrti. Prognoza bolesnika sa varijantnom anginom pektoris je nepovoljnija kod bolesnika koji imaju opstruktivne (fiksne) aterosklerotske lezije koronarnih arterija [11]. Opstruktivna koronarna bolest leči se pored medikamentne terapije i revaskularizacionim postupcima – perkutanim koronarnim intervencijama ili hirurškom revaskularizacijom.

Zaključak

Izražen i prolongiran vazospazam sa produženim prekidom protoka u koronarnoj arteriji može dovesti do pojave nekroze miokarda sa porastom aktivnosti kardiospecifičnih enzima u serumu. Bolesnici sa vazospastičnom anginom komplikovanom tranzitornim kompletnim AV blokom ili malignim srčanim tahiaritmijama imaju povećan rizik od nagle srčane smrti. Lekovi sa dilatatornim efektom na koronarne arterije – nitrati i antagonisti kalcijuma sa dugotrajnim dejstvom, dati u maksimalnim dozama, mogu uspešno prevenirati napade vazospastične angine. Statini sa antiinfalmatornim vaskularnim uticajem povoljno utiču u stabilizovanju vazospastične angine pektoris.

L I T E R A T U R A

1. *Mayer S, Hillis LD.* Prinzmetal's variant angina. Clin Cardiol 1998; 21(4): 243–6.
2. *Sueda S, Fukuda H, Watanabe K, Ochi N, Kawada H, Hayashi Y,* et al. Usefulness of accelerated exercise following mild hyperventilation for the induction of coronary artery spasm : comparison with an acetylcholine test. Chest 2001; 119(1): 155–62.
3. *Hirano Y, Uehara H, Nakamura H, Ikuta S, Nakano M, Akiyama S,* et al. Diagnosis of vasospastic angina: comparison of hyperventilation and cold-pressor stress echocardiography, hyperventilation and cold-pressor stress coronary angiography, and coronary angiography with intracoronary injection of acetylcholine. Int J Cardiol 2007; 116(3): 331–7.
4. *Sueda S, Saeki H, Otani T, Mineoi K, Kondou T, Yano K,* et al. Major complications during spasm provocation tests with an intracoronary injection of acetylcholine. Am J Cardiol 2000; 85(3): 391–4, A10.
5. *Hung MJ, Cheng CW, Yang NI, Hung MY, Cherng WJ.* Coronary vasospasm-induced acute coronary syndrome complicated by life-threatening cardiac arrhythmias in patients without hemodynamically significant coronary artery disease. Int J Cardiol 2007; 117(1): 37–44.
6. *Unverdorben M, Haag M, Fuerste T, Weber H, Vallbracht C.* Vasospasm in smooth coronary arteries as a cause of asystole and syncope. Cathet Cardiovasc Diagn 1997; 41(4): 430–4.
7. *Lip GY, Gupta J, Khan MM, Singh SP.* Recurrent myocardial infarction with angina and normal coronary arteries. Int J Cardiol 1995; 51(1): 65–71.
8. *Kijima M.* Treatment and prognosis of vasospastic angina. Nippsn Rincho 2003; 61(suppl 5): 159–66.
9. *Antman E, Muller J, Goldberg S, MacAlpin R, Rubenfire M, Tabatznik B,* et al. Nifedipine therapy for coronary-artery spasm. Experience in 127 patients. N Engl J Med 1980; 302(23): 1269–73.
10. *Tanabe Y, Itoh E, Suzuki K, Ito M, Hosaka Y, Nakagawa I,* et al. Limited role of coronary angioplasty and stenting in coronary spastic angina with organic stenosis. J Am Coll Cardiol 2002; 39(7): 1120–6.
11. *Shimokawa H, Nagasawa K, Irie T, Egashira S, Egashira K, Sagara T,* et al. Clinical characteristics and long-term prognosis of patients with variant angina. A comparative study between western and Japanese populations. Int J Cardiol 1988; 18(3): 331–49.

Primljen 13. X 2009.
Revidiran 19. II 2010.
Prihvaćen 3. III 2010.

CASE REPORT

UDC: 616-072.1::616.13/.14-089.843
DOI: 10.2298/VSP1107616S

Endovascular repair of posttraumatic multiple femoral-femoral and popliteal-popliteal arteriovenous fistula with Viabahn and excluder stent graft

Eendovaskularno zbrinjavanje posttraumatske multiple femoralnofemoralne i poplitealnopoplitealne arteriovenske fistule primenom Viabahn i ekskluder stent grafta

Momir Šarac*, Ivan Marjanović*, Miodrag Jevtić*, Sidor Mišović*, Uroš Zoranović*, Siniša Rusović†

Military Medical Academy, *Clinic for Vascular Surgery, †Institute of Radiology, **Belgrade, Serbia**

Abstract

Background. Traumatic arteriovenous (AV) fistula is considered to be a pathologic communication between the arterial and venous systems following injury caused mostly by firearms, sharp objects or blasting agents. Almost 50% of all traumatic AV fistulas are localized in the extremities. In making diagnosis, besides injury anamnesis data, clinical image is dominated by palpable thrill and auscultator continual sounds at the site of fistula, extremities edemas, ischemia distally of fistula, pronounced varicose syndrome, and any signs of the right heart load in high-flow fistulas. **Case report.** We presented a male 32-year-old patient self-injured the region of the right lower and upper leg by shotgun during hunting in 2005. The same day the patient was operated on in a tertiary traumatology health care institution under the diagnosis of *vulnus sclopetarium femoris et cruris dex*; AV fistula *reg popliteae dex*; *fractura cruris dex*. The performed surgery was *ligatura* AV *fistulae*; *reconstructio a. popliteae cum* T-T anastomosis; *fasciotomia cruris dex*. Postoperatively, in the patient developed a multiple AV fistula of the femoral and popliteal artery and neighboring veins. The patient was two more times operated on for closing the fistula but with no success. Three years later the patient was referred to the Clinic for Vascular Surgery, Military Medical Academy, Belgrade, Serbia. A physical examination on admission showed the right upper leg edema, pronounced varicosities and high thrill, signs of the skin induration and initial ischemia with ulceration in the right lower leg, as well as numerous scars in the inner side of the leg from the previously performed operations. Due to the right heart load there were also present easy getting tired, tachypnoea and tachycardia. CT and contrast angiography verified the presence of multiple traumatic AV fistulas in the surface femoral and popliteal artery and neighboring veins of the highest diameter being 1 cm. Also, numerous metallic balls – grains of shotgun were present. After the preoperative preparation under local infiltrative anesthesia, transfemoral endovascular reconstruction was done of the surface femoral and popliteal artery by the use of stent grafts Viabahn 6 × 50 mm and excluder PXL 161 007. Within the immediate postoperative course a significant reduction of the leg edema and disappearance of thrill occurred, and, latter, healing of ulceration, and disappearance of signs of the foot ischemia. Also, patient's both cardiac and breathing functions became normal. **Conclusion.** In patients with chronic traumatic AV fistulas in the femoropopliteal region, especially with multiple fistulas, the gold standard is their endovascular reconstruction which, although being minimally traumatic and invasive, offers a complete reconstruction besides keeping integrity of both distal and proximal circulation in the leg.

Key words:
arteriovenous fistula; leg injuries; therapeutics; vascular surgical procedures; stents.

Apstrakt

Uvod. Smatra se da je traumatska arteriovenska (AV) fistula patološka veza između sistema arterija i vena nastala posle povrede izazvane vatrenim oružjem, oštrim predmetima ili eksplozivnim sredstvima. Gotovo 50% svih AV fistula nalazi se na ekstremitetima. Pri postavljanju dijagnoze, osim istorije povrede, kliničkom slikom dominiraju palpabilni tril, auskultatorno neprekidni zvuci na mestu fistule, edemi ekstremiteta, ishemija distalno od fistule, izraženi varikozni sindrom, kao i bilo koji znak opterećenja desnog srca kod visokoprotočnih fistula. **Prikaz bolesnika.** Prikazali smo bo-

Correspondence to: Momir Šarac, Military Medical Academy, Clinic for Vascular Surgery, Crnotravska 17, 11 000 Belgrade, Serbia. Phone: +381 11 3608 570.

lestnika, u dobi od 32 godine, koji se samoranio u desnu nogu 2005. godine u lovu. Istog dana bolesnik je operisan u traumatološkoj zdravstvenoj ustanovi tercijalnog nivoa sa dijagnozom *vulnus sclopetarium femoris et cruris dex*; AV fistula *reg popliteae, fractura cruris dex*. Urađena je operacija ligatura AV fistule, *reconstructio a. popliteae cum* T-T *anastomosis;* fasciotomija *cruris dex*. Postoperativno, kod bolesnika razvila se multipla AV fistula u zoni femoralne i poplitealne arterije i okolnih vena. Bolesnik je još dva puta operisan zbog zatvaranja fistule, ali bez uspeha. Tri godine posle povrede bolesnik je upućen u Kliniku za vaskularnu hirurgiju Vojnomedicinske akademije, Beograd, Srbija. Na pregledu, prilikom prijema uočen je edem desne butine, upadljive proširene vene i tril, znaci induracije kože i početna ishemija sa ulceracijama na desnoj potkolenici, kao i mnogobrojni ožiljci sa unutrašnje strane noge, od prethodnih operacija. Zbog opterećenja desnog srca dolazilo je do lakog zamora, tahipneje i tahikardije. Na CT i kontrastnoj angiografiji potvrđeno je prisustvo multiplih traumatskih AV fistula na površnoj femoralnoj i poplitealnoj arteriji i okolnim venama, sa najvećim prečnikom od 1 cm. Takođe, bile su prisutne i mnogobrojne metalne kuglice – lovačka sačma. Posle preoperativne pripreme pod lokalnom infiltrativnom anestezijom izvršena je transfemoralna endovaskularna rekonstrukcija površne femoralne i poplitealne arterije primenom stenta Viabahn 6 × 50 cm i ekskludera PXL 161 007. U neposrednom postoperativnom toku došlo je do znatnog smanjenja edema noge i prestanka trila i, kasnije, izlečenja ulceracija i nestanka znakova ishemije stopala. Takođe, normalizovali su se srčana i disajna funkcija. **Zaključak.** Kod bolesnika sa hroničnim traumatskim AV fistulama u femoropoplitealnoj regiji, naročito sa multiplim fistulama, zlatni standard predstavlja njihova endovaskularna rekonstrukcija koja, kao minimalno traumatska i invazivna, omogućava ne samo kompletnu rekonstrukciju, već i očuvanje integriteta i distalne i proksimalne cirkulacije noge.

Ključne reči:
arteriovenska fistula; noga, povrede; lečenje; hirurgija, vaskularna, procedure; stentovi.

Introduction

Traumatic arteriovenous (AV) fistula is a pathologic communication between arterial and venous blood flow due to a penetrating injury, mostly caused by firearms, sharp objects or blasting agents [1, 2]. Also, AV traumatic fistula could be caused by fracture of other bones of extremities when sharp fragments of bones lead to laceration of neighboring vascular structures [3, 4], sport activites [5, 6], and, less frequently, after orthopedic injures [7–9].

To make the diagnosis of traumatic AV fistula, except for adequate anamnesis and physical examination it is required to do color duplex scan, CT angiography, as well as classical angiography. It is significant to note that these diagnostic procedures do not exclude one another, but, on the contrary, they complement each other, and help in making a final plan for the treatment method of patients with traumatic AV fistula [10].

The therapeutic strategy before the endovascular era included artery reconstruction or ligation with autologous material, external compression [11, 12], coil embolization, or echo-guided thrombin injection [13, 14].

Parodi et al. [15] and Marin et al. [16] have described the treatment of complex peripheral lesions with satisfactory results for arterial injuries of the arms and the neck.

Case report

A 32-year-old patient referred to the Clinic for Vascular Surgery, Military Medical Academy (MMA), Belgrade, Serbia due to additional diagnostics and surgical treatment of traumatic AV fistula in the right lower limb.

The patient had himself-injured by accident during hunting three years before. Then the patient was primary managed in another institution under the diagnosis: *Vulnus sclopetarium femoris et cruris dex*; AV fistula of the right lower extremites; Fracture of the right tibia. The then performed operation was: ligature AV fistula; reconstruction of popliteal artery with end to end anastomosis; *fasciotomia*.

After that, the patient was hospitalized two more times and operated in specialized vascular surgery institution. Due to the presence of traumatic AV fistula in the right lower limb the patient was firstly operated: ligature of multiple AV fistula transvenosum. Next, due to further presence of AV fistula in the right lower and upper limb regions the surgery was performed new operation: ligature AV *fistulae et ligature posterior tibialis artery*. The patient was then dismissed from the hospital with a further verified presence of traumatic multiple AV fistula in the right leg.

At admittance to the Clinic for Vascular Surgery of MMA, the patient was in the vascular condition as follows: the right leg extensively voluminous, almost of two times higher circumference than the left leg, with the presence of palpable thrill and auscultator sounds along the whole upper limb and the dilated surface veins of the upper limb. The pulse was weaken, in the anterior tibial artery, in the right foot, while pulse posterior tibial artery was not palpable at all. The skin in the right lower limb was dark, indurate, having a blistering ulceration at the medial side. The foot was cool, livid – discolorated. The patient got tired after a few steps, showing the signs of tachycardia and tachypnoea. A radiogram showed the presence of cardiomegaly in the right heart.

The performed diagnostic procedures, color duplex scan, MSCT angiography and classical angiography confirmed the presence of multiple post-traumatic AV fistula within the area of distal third of the right superficial femoral artery, as well as the popliteal artery and the neighboring deep veins (Figures 1 and 2). The largest of the fistula had the diameter of about 10 mm, while many of them up to 2 mm both distally and proximally from it. There was also dilatation of the superficial femoral artery above the site of the most proximal fistula, having the diameter of about 12 mm at that site, as well as the occlusion of the tibial posterior

artery. The popliteal artery diameter below the site of the most distal fistula was 5 mm. In the soft tissues of the upper and lower leg, and in the knee joint there were numerous foreign bodies – metallic balls (lead rounds).

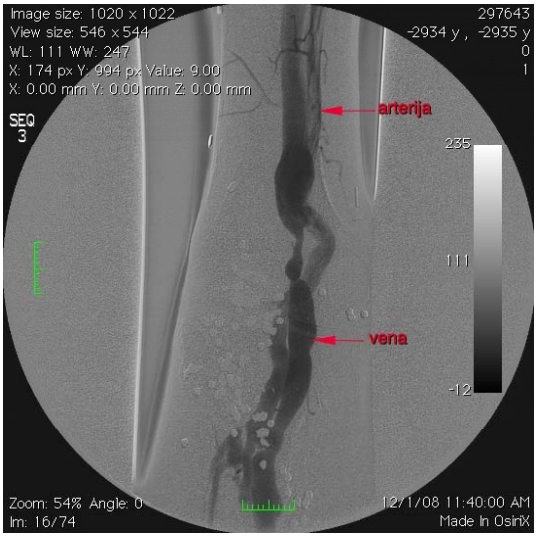


Fig. 1 – The artery and veins with multiple arteriovenous (AV) fistulas

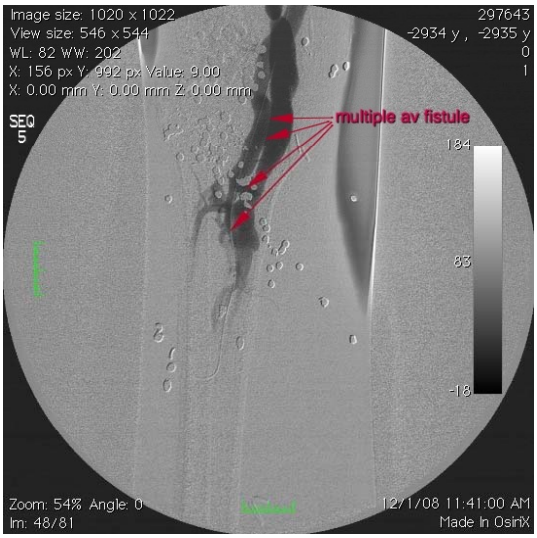


Fig. 2 – Multiple arterivenous (AV) fistulas

After the adequate preoperative procedure, with local infiltrative anesthesia and transfemoral approach under radiography control endovascular surgery was performed: endovascular reconstruction of superficial femoral artery and popliteal artery with Viabahn and excluder stent graft.

After local anesthetic administration (2% xylocain 20 mL) in the right femoral area, firstly was done open approach to the right common femoral artery to a typical site. Then, puncture was made to the artery complete with placing a catheter for angiography to identify a site of most proximal and most distal AV fistula (Figure 2). The first to place was a Viabahn stent graft 6 × 50 mm through a dilatators of 7 Fr, and after that was placed excluder-iliac extender stent graft PXL 161007 through a dilatator of 12 Fr with overlapping of the two stents for 1 cm so that the distal portion of the excluder was placed in the proximal part of a viabhan stent graft (Figure 3). Each of the placed stent grafts were of Gore-Tex production.

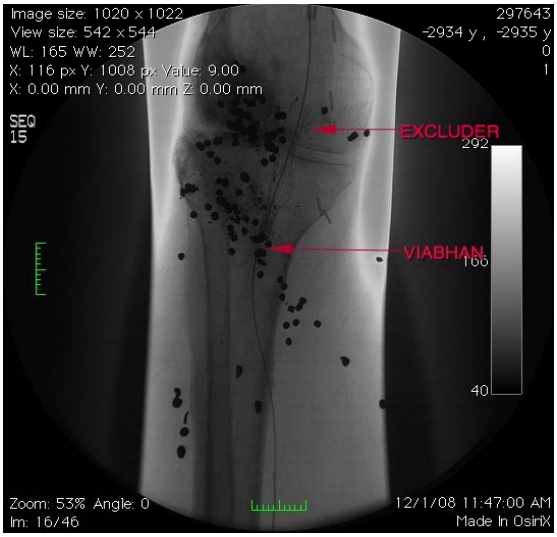


Fig. 3 – The placed stent grafts

A control angiography showed a complete reconstruction of superficial femoral artery and popliteal artery, as well as transient anterior tibial artery up to the foot, while the deep and superficial venous system was not presented – there is no sings of AV fistula (Figure 4).

Fig. 4 – The reconstructed arteries (superficial femoral artery and popliteal artery)

Nearly after the surgery a significant reduction of edema of the leg occurred and disappearance of thrill and auscultator sound, and, later, initial healing of ulceration started and disappearance of the signs of the foot ischemia (Figure 5).

Fig. 5 – The leg after surgery

Control examination after six month (including CT angiografy) singed that there was no more AV fistulas (Figure 6). The right leg was absolutely with normal function.

Fig. 6 – The knee: X-ray image after the surgery

Discussion

In the era of daily increase in traffic traumas and peacetime injuries with firearms and side arms, there are more often injuries of the vascular structures, especially lower extremities. As complication of the said due to inadequate open surgical reconstruction of the blood vessels there appear traumatic AV fistulas. Any next surgery, the so-called redo operation, is especially difficult due to fibroses tissue that forms around blood vessels after previous interventions. Also, there is a possibility of disturbing integrity of walls of the blood vessels provoking further complications [2].

The incidence of AV fistulas in blood vessels injuries ranges from 2.3% to 3.9% according to the published large series worldwide. According to the Vietnam Vascular Register, out of 7 500 war injuries of blood vessels 262 patients had traumatic AV fistulas that is 3.5% [1]. The incidence of AV fistulas in civilian vascular injuries was similar: 6 (2.3%) of 256 injuries in a review by Patman et al. [17] and 7 (3.6%) of 192 patients in a review by Sirinek et al [18]. The most frequent localization of traumatic AV fistula are lower extremities, in almost 50% of cases, out of which injuries of femoral blood vessels with AV fistulas are present in 17% of cases [2, 16]. The second place is taken by the region of the neck with carotid blood vessels, and then the head (carotid-cavernous fistulas) [17].

Clinical image of traumatic AV fistula in the extremities is dominated by the presence of palpable thrill and auscultation systolic-diastolic sounds in the skin above a fistula, as well as a visible scar from injury. Then, there is a pulsate mass, dilated surface veins of the extremities, weaken artery pulse distally to the site of fistula, edema of the extremities, while in chronic fistulas there is a possibility of skin indurations, ulceration, even ischemia of the extremities followed by gangrene. Also, there is a possibility of positive Branham-Nicoladoni sign: manual compression to the artery prior to a fistula leads to arterial excitement of baroreceptors due to sudden reduced inflow of blood in the right heart, and, thus, to the subsequent bradicardia and hypotension [1, 2, 19].

In the literature, the combined endovascular treatment of AV fistula has been reported for different large arterial territories, including iliac, subclavian, and carotid arteries. Good early and midterm results have been described. To treat pseudoaneurysms or AV fistulas of the popliteal artery, the surgical approach has been preferred even recently, with direct ligation of the injured vessel or reconstruction of the vessel wall [2].

Following the development of new technological and technical accomplishments in vascular surgery, including stent grafts, solving that kind of problems is possible by the use of endovascular approach. It means that a site of problem in the blood vessel (AV fistula, aneurysm) is approached through the blood vessel itself, but at the site at which it is not damaged, thus avoiding approach through scary tissue [5, 20]. So, endovascular reconstruction of a blood vessel is minimally invasive and traumatic to a patient. Endovascular reconstruction itself requires determination of appropriate stent size to be placed in accordance with a precise determination of blood vessel size through CT angiography. It is primarily referred to the necessity to fix a stent graft in its distal and proximal part to a healthy wall of the artery (so-called neck) along minimally 10 mm in order to prevent migration of a stent graft.

The current problem is an unavailable conus stent graft for major arteries that is stent grafts with lumen diameter that uniformly reduces from proximal to distal end. This problem we solve by simultaneous application of both, excluder iliac extender and Viabahn stent graft (made by Gore-Tex) by interposing one in another. Furthermore, as innovations in stent technology evolve, there will probably be more flexible, longer, and less thrombotic and immunogenic stents available in the near future.

Conclusion

In patients with post-traumatic AV fistulas in the femoral-popliteal or another region, particularly in multiple fistulas and redo operation endovascular treatment is safe, simple, and less traumatic. Procedure should be applied when it is technically feasible, taking into account the findings as well as local extensiveness of AV fistulas.

Endovascular treatment provides a complete reconstruction and maintaining integrity of both distal and proximal blood flow in the extremities.

Consequently, the success rate and efficacy of endovascular treatment of peripheral AV fistulas and pseudoaneurysms shall rise.

R E F E R E N C E S

1. *Rich NM, Spencer FC.* Arteriovenous fistulas. *In*: *Rich NM, Spencer FC*, editors. Vascular trauma. Philadelphia: WB Saunders Company; 1978. p. 191–232.
2. *Rich NM, Hughes CW*. Vietnam vascular registry: a preliminary report. Surgery 1969; 65(1): 218–26.
3. *Ilijevski NS, Nenezić DD, Sagić D, Radak D*. Popliteal traumatic arteriovenous fistula. Vascular 2005; 13(5): 309–12.
4. *Ward AS, Carty NJ, O'Rourke NA.* Arteriovenous fistulae complicating orthopaedic procedures. Aust N Z J Surg 1995; 65(12): 905–7.
5. *Rosa P, O'Donnell SD, Goff JM, Gillespie DL, Starnes B.* Endovascular management of a peroneal artery injury due to a military fragment wound. Ann Vasc Surg 2003; 17(6): 678–81.
6. *Bandy WD, Strong L, Roberts T, Dyer R.* False aneurysm-a complication following an inversion ankle sprain. J Orthop Sports Phys Ther 1996; 23(4): 272–9.
7. *Skudder PA, Gelfand ML, Blumenberg RM, Fulco J*. Tibial artery false aneurysm: uncommon result of blunt injury occurring during athletics. Ann Vasc Surg 1999; 13(6): 589–91.
8. *Mureebe L, Gahtan V, Kahn MB, Kerstein MD, Roberts AB*. Popliteal artery injury after total knee arthroplasty. Am Surg 1996; 62(5): 366–8.
9. *Chervu A, Quinones-Baldrich WJ*. Vascular complications in orthopedic surgery. Clin Orthop Relat Res 1988; 235: 275–88.
10. *Maxwell-Armstrong CA, Taylor AM, Majkowski RS, Colton CL*. False aneurysm of the anterior tibial artery following removal of tibial plate. Eur J Vasc Endovasc Surg 1995; 10(4): 505–6.
11. *Wolford H, Peterson SL, Ray C, Morgan SJ*. Delayed arteriovenous fistula and pseudoaneurysm after an open tibial fracture successfully managed with selective angiographic embolization. J Trauma 2001; 51(4): 781–3.
12. *Edwards H, Martin E, Nonygrod R*. Nonoperative management of a traumatic peroneal artery false aneurysm. J Trauma 1982; 22(4): 323–6.
13. *Hertz SM, Brener BJ*. Ultrasound-guided pseudoaneurysm compression: efficacy after coronary stenting and angioplasty. J Vasc Surg 1997; 26(6): 913–6.
14. *Kang SS, Labropoulos N, Mansour MA, Michelini M, Filliung D, Baubly MP,* et al. Expanded indications for ultrasound-guided thrombin injection of pseudoaneurysms. J Vasc Surg 2000; 31(2): 289–98.
15. *Parodi JC, Schönholz C, Ferreira LM, Bergan J*. Endovascular stent-graft treatment of traumatic arterial lesions. Ann Vasc Surg 1999; 13(2): 121–9.
16. *Marin ML, Veith FJ, Panetta TF, Cynamon J, Sanchez LA, Schwartz ML*, et al. Transluminally placed endovascular stented graft repair for arterial trauma. J Vasc Surg 1994; 20(3): 466–72.
17. *Patmen RD, Poulos E, Shires GT*. The management of civilian arterial injuries. Surg Gynecol Obstet 1964; 118: 725–38.
18. *Sirinek KR, Gaskill HV 3rd, Dittman WI, Levine B.* Exclusion angiography for patients with possible vascular injuries of the extremities: a better use of trauma center resources. Surgery 1983; 94(4): 598–603.
19. *McNeil JD, Chiou AC, Gunlock MG, Grayson DE, Soares G, Hagino RT*. Successful endovascular therapy of a penetrating zone III internal carotid injury. J Vasc Surg 2002; 36(1): 187–90.
20. *Baltacioğlu F, Cimşit NC, Cil B, Cekirge S, Ispir S*. Endovascular stent-graft applications in latrogenic vascular injuries. Cardiovasc Intervent Radiol 2003; 26(5): 434–9.

Received on July 5, 2010.
Revised on Avgust 5, 2010.
Accepted on September 1, 2010.

H I S T O R Y O F M E D I C I N E

UDC: 75-051::355.415.6(497.11)"1914/1918"(091)

Serbian painters in the Army Medical Corps 1914–1918

Srpski slikari u ratnom sanitetu 1914–1918

Ljubiša Nikolić

Clinical Centre "Zvezdara", Clinic for Eye Diseases, **Belgrade, Serbia**

Key words:
world war I; serbia; military medicine; famous persons.

Ključne reči:
prvi svetski rat; srbija; medicina, ratna; slavne ličnosti.

Introduction

Searching documents in the history of the Serbian Army Medical Corps reveals their different fortune: while the Serbian-Turkish Wars (1876–1878), and the Serbian-Bulgarian War were described by the hand of Dr. Vladan Đorđevic and published in four volumes [1], a larger part of the archive which could shed more light on the Great War was destroyed either in trains at the town of Kraljevo railway station or at the torches lit at Kosovo and Metohia, during the retreat of the Serbian Army in 1915. Fortunately, there are other sources as memoires and the medical papers of those who participated in the wars from 1912–1918, gathered and published by Dr. Vlada Stanojević [2], a book written by a Polish volunteer, Prof. Dr. Ludvig Hirszfeld [3], and Dr. Svetislav Barjaktarović's war diary [4]. Recently, a group of authors has reconstructed the work of the Medical War Corps based on the survived documents and other sources [5, 6]. Not long ago, the life and and tragic death of Nadežda Petrović, a voluntary nurse in two wars, a great painter, and Serbian heroine was presented [7]. Nadežda's work and death from epidemic typhus fever have been widely known to the general public: an art gallery and an exhibition, a monument, and her portrait on a bank-note all keep the memory of this artist, philanthropist and patriot. However, the triumph of Serbia in the Great War had its price – sufferings of the whole people, including a complete generation of the young Serbian painters*, Nadežda's colleagues and pupils, whose names are known mainly to the art circles. Yet, most of them were either voluntary nurses or patients of the Army Medical Corps, or both, from 1914 to 1918. Therefore, the aim of this paper was to make them known to the medical community.

Although with a different human and artistic nature, these young men and women, as a generation, followed the same path. At first, they would attend the art school in Belgrade, lead by Kiril Kutlik, and later, by Rista and Beta Vukanović.

After having learnt the basic drawing, they would switch to Anton Ažbe's preparatory art school in Munich. Later, in the first decade of the 20th century, they would graduate from the Fine Arts Academy in that German city. A touch of French impressionism would make some of them go to Paris in order to get acquainted with this revolutionary style more closely, while others would return to Serbia and try to excercise impressionism while working as school teachers.

Most of them were in the wars 1912–1918: women as the voluntary nurses, men as the war painters or soldiers. Unfortunately, they reached the peak of their art expression in the time of war. On the other hand, places where they were sent to gain strength after a life-threatening disease, the Mediterranian islands of Corfu and Capri, were a paradise for an impressionist's eye, and set the scene for some of the capital achievements of Serbian art. As the war painters, these young men produced drawings and the small size paintings of a documentary value. Contrary to many works of the western art of that time, the scenes which they depict rarely show hopelessness and horror, and never send an anti-war message.

The post-war exhibitions of their paintings in 1919 and 1922 were followed by a tide of expressionist art embraced by yet a younger generation of painters who had little respect for impressionism. Except for a very few, the whole generation of our impressionists by style, and nurses or war painters out of the patriotic obligation, had been forgotten, and brought back from the oblivion decades later [8].

* Painters from Serbia and volunteers (V), who took part in the war but escaped from injuries or disease, are listed here: Vladimir Becić (V), Nikola Bešević (V), Bogosav Vojnović Pelikan, Vilko Gecan (V), Miloš Golubović, Oton Iveković (V), Nikola Jeremić, Živojin Lukić, Mihailo Milovanović, Stevan Milisavljević, Dragoljub Pavlović, Branko Popović, Stevan Stanković, Radomir Stevanović, Hristifor Crnilović. Ignjat Job and Todor Švrakić were imprisoned by the Austrian authorities.

Correspondence to: Ljubiša Nikolić, Clinical Centre "Zvezdara", Clinic for Eye Diseases, Dimitrija Tucovića 161, 11 000 Belgrade, Serbia. Phone: +381 11 241 4931. E-mail: nikolic_ljubisa@yahoo.com

The eldest in this generation, Beta Vukanović and Nadežda Petrović had voluntarily joined the Army Medical Corps as nurses much earlier – in the Balkan Wars 1912–1913, while Dragomir Glišić and Petar Ranosović became the first appointed war painters ever [9, 10] †. Glišić, who was a life-long teacher of art, joined the Danube Division of our Army in 1914. In Thessaloniki, he became a war painter and photographer.

A year later, the first victims of the World War tempest fell: Danica Jovanović was accused of Serbian patriotism, and executed at Petrovaradin Fortress, while Aleksandar-Šaca Jovanović and Cvijeto Job, a volunteer, lost their lives in combat [11–13].

The disease was stronger than the efforts of the Medical Corps to save Mališa Glišić's and Nadežda Petrović's life, and they both died in 1915. Mališa's studies in Munich were more a fight with poverty, contrary to his Italian period (1911–1912), marked with large, pastously painted landscapes full of light and colour, and exhibited in the Serbian Pavilion at the World Art Show in Rome. This ended in the Balkan wars, where he was a war painter, and in the Great War, which cut his life at the age of 29. The following year, Branko Jevtić, from Belgrade, was killed in a battle near Bitola, leaving only the unfinished paintings to remind us of his talent. Branko Radulović from Mostar, a volunteer in two wars, and one of the best Bosnian and Herzegovinian impressionist painters, died in Skopje. At the end of the war, the elderly Rista Vukanović and seriously wounded, young Kosta Josipović succumbed, too [11–13].

Voluntary nurses in the Army Medical Corps

Beta Vukanović, Nadežda Petrović, Mara Lukić-Jelesić, Ana Marinković, Jelica-Jela Marković, and Natalija Cvetković were voluntary nurses in the Great War.

Beta Vukanović (Bamberg, Germany, 1872–Belgrade, 1972) had attended the art schools in Munich and Paris. Born as Babette Bachmayer, she had married Rista Vukanović, and shared the horrors of the wars from 1912–1945 with her new compatriots. Beta had influenced Serbian art in many ways: as a painter, a teacher and a founder of art societies. As early as in 1900, together with her husband, she founded a School of Fine Arts in Belgrade. The beginnings of Lada Art Society, and the Association of Artists have firmly been connected with her name. Her retrospective exhibition was held in Belgrade in 1958 [11–14].

Nadežda Petrović (Čačak, 1873–Valjevo, 1915) started as a pupil of Kutlik's in Belgrade. Later, she went to Munich (1902) to study under Anton Ažbe and Julius Ekster, and to paint "in a manner that would reappear some years later, in the same place, in the work of Kandinsky and the Blaue Reiter Expressionists" [13]. Nadežda was ahead of her time, both in her style and in her vigorous efforts to introduce Modernism. She exhibited at the First Yugoslav Art Exhibition in 1904, next to her admired Jakopič, Grohar, Jama and Sternen. The following year she founded the first Serbian artists' colony at Sićevo. Finally, she left for Paris (1910–1912), where she met the Fauves, and exhibited at the famous Autumn Salon. When the war of the Balkan allies against Turkey started, she returned to Serbia to volunteer as a nurse in the Army Medical Corps, and served from one war to another, until her untimely death from epidemic typhus fever. Her retrospective exhibition was held in the Museum of the Contemporary Art in Belgrade in 1973 [11].

Mara Lukić-Jelesić (Novi Kneževac, 1885–Šabac, 1979) studied under Beta and Rsita Vukanović and later in Munich. After volunteering as a nurse in the First World War, she was a school teacher of art [11].

Ana Marinković (Belgrade, 1881–Getari, France, 1973) had studied under Nadežda Petrović and Beta Vukanović before she went to Paris and London to attend private art schools. As a voluntary nurse, she helped the diseased and wounded soldiers through Albanian mountains, all the way to Corfu. Her first exhibition was with Lada Society in 1910 [11, 13].

Jelica-Jela Marković (Belgrade, 1891–Belgrade, 1969) took lessons from Beta Vukanović. She volunteered as a nurse as long as the Medical Corps acted on Serbian teritory. After the war, she chose another occupation, and her paintings have been kept within her family [14].

Natalija Cvetković (Smederevo, 1888–Belgrade, 1928) attended the Vukanović School of Fine Arts in Belgrade, and afterwards studied in Munich and Paris. During the war, she volunteered as a nurse. She was one of Lada's founders, and exhibited at the First Yugoslav Art Show in 1904. Her retrospective exhibition was held in the Museum of Contemporary Art in Belgrade in 1974 [11–13].

Convalescents

Infective diseases were yet another enemy of Serbian soldiers, officers, and the members of the Army Medical Corps. A vivid picture of epidemic typhus fever and the conditions in which the Corps had to act, has been brought to us by the War Diary of Dr. Svetislav Barjaktarović. No statistics can match these short notes written on the spot, even at the height of the author's disease‡. Those lucky

†While the British Army did not encourage artists to make drawings of the battlefield scenes in the Great War fearing that the enemy could obtain information from them, our Staff had introduced the term "war painter" as early as 1912. This title appeared in the Travel permission number 86 signed by Colonel Živojin Mišić, and issued to Petar Ranosović, as well as in the Permission number 4190 issued to Dragomir Glišić according to the Order signed by Petar Bojović, Chief of Staff. Many artists who bore this title in the Great War left valuable eyewitness documents [11].

‡P. 67. " 1915. Jan. 11. Vlaška. Mud and humidity in this village are huge. Water has destroyed most of the huts. There are many cases of trench fever, as well as of epidemic typhus fever and pox. Feb.1. Vlaška. There are about 200 patients in this hospital, all suffering from infective diseases: *Febris exantematica, Febris recurrens, Variolla vera*. I asked to work in this hospital in order to get acquainted with epidemic typhus fever and trench fever. Number of patients with epidemic typhus fever is 32, and 70 suffer from trench fever." P. 69. " Feb. 12. Vlaška. Today, I feel fever. 37.3ºC. Feb. 13. Vlaška. Today, I stayed in bed. 38ºC. But, I got up in the evening, as I felt better, and my temperature dropped. Feb. 14. Vlaška. Illness is overcoming me, fever is raising, I feel worse. Feb. 15. Vlaška. I was moved to share the room with Dr. Andra, Đura, and major Gvozden Ristanović. This means that my disease is epidemic thyphus fever".

enough to survive the disease were sent to recover at the hospitals along the Mediterranean coast. Veljko Stanojević (Belgrade 1892–Belgrade 1967) was an exception: he was transferred from a combat division to the Department of Topography. Stanojević had studied under Marko Murat and Ljuba Ivanović before the war, and in Paris after the war. He participated in the group exhibitions in 1922, 1927, and 1940. His retrospective exhibition was held in 1957 in Belgrade [11].

Two painters were treated either in Russian or Austrian hospitals: Vasa Pomorišac (Modoš, 1893–Belgrade, 1961), a volunteer, was hospitalized in Russia after being badly wounded at Dobrudža. He put an immense effort into joining our Army in Thessaloniki, after the travel through Siberia, Mancuria, and across the Indian Ocean [11].

Nikola Džanga (Belgrade, 1892–Belgrade, 1960) was dangerously wounded near Aleksandrovac in 1915, and immediately sent to captivity in Austria [11].

Živorad Nastasijević (Gornji Milanovac, 1893–Belgrade, 1966), ill and with bad contusions from a battle for Kaimakchalan, was transported to the hospital in Thessaloniki, and further to Algeria, as a convalescent. He graduated from the Art School in Belgrade, and studied at the Academy in Munich. He spent a part of the war in battle forces, and later became a war painter. After the war, he studied in Paris. Nastasijević exhibited at the War Painters Show in 1919, and was one of the founders of the Zograph group [11, 12].

Vasa Eškićević (Irig, 1876–Novi Sad, 1933) who had studied historical painting in Sankt Petersburg under Ilya Riepin, joined our Army as a volunteer, to become a war painter. He convalesced in Rome and Paris, but there are no available documents to show the nature of his illness or injuries [11].

The same holds true for Josip-Sibe Miličić (Brusje, Hvar–Bari, 1945) whose convalescence took part at Capri and in France [12].

Dragoslav Vasiljević-Figa (Kragujevac, 1895–Kruševac, 1929) spent his convalescence near Biserta, where his duty was to create scenography for an army theatre. Figa's art education began in 1911, under Rista and Beta Vukanović, and ended after the war. His exhibitions were held in Priština (1920), and in Belgrade, 1975 (retrospective) [11–13].

Vlastimir Petrović (Dubravica, 1888–Belgrade, 1950) was sent to Sidi Abdalah in Tunisia to recover from malaria. There, he painted icons for a military chapel and the theatre scenography. His numerous drawings with extensive notes, including dialogues, are a living memento of the war times. Petrović had attended art schools in Belgrade and Munich before the war, and in Paris, after the war. Later, he became a high-school teacher of art. His one-man exhibition was held in 1926, and a retrospective was in 1963, in Belgrade [11–13].

Paško Vučetić (Split, 1871–Belgrade, 1950) spent his convalescence at Corfu, and later in Italy, where he attended art schools in Trieste and Venice (later in Munich, Germany) at the end of the 19th century, and exhibited in Trieste in 1901. Although elder than most of the war painters, Paško accepted their style and painted vedutes of Rome in 1916, which shine with orange buildings, blue sky and violet shades. His art also included sculpure and copying fresco paintings (as a member of the staff of the National Museum in Belgrade). Vučetić had assisted Nadežda Petrović in organizing the First Serbian Artists' Colony [11–14].

Kosta Miličević (Vraka, 1877–Beograd, 1920) was recovering at Corfu twice: in 1916–1917, and in 1918. In spite of that, he was so weak that he succumbed to the Spanish fever in 1920. He studied painting under Kutlik in Belgrade, Ažbe in Munich, in private schools in Vienna, and again in Belgrade. Kosta's original technique which included short brush strokes and a thick paint, as well as his charisma, made him an admired leader of a group of young Belgrade impressionists. His almost musical treatment of two motives: St. Sava's church before the war, and the landscapes of Corfu during the war, places him, together with Nadežda Petrović and Milan Milovanović, among the three most prominent Serbian artists of that time. A retrospective exhibition of his works was held in the Museum of Contemporary Art in Belgrade in 1973-4 [11–15].

Fig. 1 – Milan Milovanović – Drawing (pencil on paper), C 1249; National Museum, Belgrade

Milan Milovanović (Kruševac, 1876–Belgrade, 1946) recovered from epidemic typhus fever at Capri during 1917–1918. Young Milovanović was lucky to live in a country which had a Minister of education, a famous academician Mihailo Valtrović, who assumed that his duty was to tour even the remotest Serbian schools in a search for gifted youth. This is how Milan, a high school student from Kruševac, got a state scholarship for studies abroad, which

lasted from 1895 to 1905: first under Kutlik in Belgrade, then under Ažbe in Munich who prepared him to graduate from the Academy in Munich, study at the Colarossi Private Academy in Paris and to graduate from the famous L'Ecole des Beaux Arts in that city! He paid his country back immediately, in 1907, with a long official tour of monasteries in Serbia, Macedonia, and on the Mountain Athos in Greece, when he painted one of the earliest and purest impressionist paintings of Serbian art – „ The Bridge of the Tsar Dušan in Skopje“ [16, 17]. However, his achievement as a war painter stays within the limits of a document [18]. According to M. Đurić, views of the battle field, narrowed by the frame of opservation post, with mixed roars of cannons and of the wounded, suited neither a sensitive artistic nature nor an impressionist painter [17].

Fig. 2 – Milan Milovanović – Drawing (pencil on paper), C 1256; National Museum, Belgrade

Chiaro of Capri, where the shapes vibrate and the shades hide, was such a revelation for Milovanović, broken by illness and left with the damaged nerves and with a hearing loss, that he painted there a series of masterpieces which rank second to none in Serbian landscape art (Figure 3).

Fig. 3 – Milan Milovanović – The Blue Door, 1917 (oil on canvas, 480 × 390 mm); National Museum, Belgrade

After the war, he continued to paint for two more years, choosing either scenes from the epic past, or views of Dubrovnik, almost as good as those of Capri. In 1920, at the age of 45, and after only a decade of a full artistic activity, he chose to live in a creative silence, painting a routine portrait or a flower arrangenent from time to time. Until his death in 1946, he was teaching at the School (later, Academy) of Fine Arts. Retrospective exhibitions were held in the National Museum in Belgrade (1960), and at Kruševac (1963, 1966) [11–13, 16–18].

Conclusion

Serbian painters had a significant role in the Army Medical Corps in the Great War. It is tempting to imagine what direction Serbian art would have taken, had Nadežda Petrović, Mališa Glišić, Kosta Miličevićvić, and their numerous friends stayed alive, and Milan Milovanović continued to paint.

Acknowledgement

I am greatly indebted to Dr. Irina Subotić, Jelena Dergenc, and to the National Museum, Belgrade for their kind help.

R E F E R E N C E S

1. *Đordević V*. History of the Serbian medical corps. I-IV. Belgrade: Državna štamparija; 1879, 1880, 1886, 1893. (Serbian)
2. *Stanojević V*. History of the Serbian medical corps. Belgrade: Vojnoizdavački i novinski centar; 1925. (Serbian)
3. *Hirszfeld L*. A Life History. Belgrade: Srpska književna zadruga; 1962. (Serbian)
4. *Nikoliš G*. The War Diary of Dr. Svetislav Barjaktarović. Beograd: SANU (posebna izdanja knjiga DLXXIII, Odeljenje medicinskih nauka knjiga 35); 1987. (Serbian)
5. *Nedok A*. The retreat of Serbian Army towards Albanian litoral and its evacuation to Corfu 1915-1916. The work of the Army Medical Corps. Belgrade: Stubovi kulture; 2008. (Serbian)
6. *Nedok A*. The Serbian Army Medical Corps in 1917-1918. Vojnosanit Pregl 2008; 65(Suppl 1): 19–26. (Serbian)
7. *Babić RR*. Nadezda Petrović-a female painter and a nurse. Vojnosanit Pregl 2008; 65(10): 783–6. (Serbian)
8. *Vasić P*. Forgotten Serbian Impressions – Mališa Glišić. Revija, Beograd 15.II. 1953. (Serbian)

9. History Museum of Serbia. Inv. no 2416. (Serbian)
10. Military Museum. Inv. no 11440. (Serbian)
11. *Vasić P, Šuica N*. Works 1912–1918. Catalogue of the Art Collection, Belgrade: Military Museum, 1982. (Serbian)
12. *Trifunović L*. The War Painters 1912–1918. Beograd: Narodni muzej-Vojni Muzej; 1964. (Serbian)
13. *Živković S*. Belgrade impressionists. Belgrade: Zlatousti; 2004. (Serbian)
14. *Živković S*. Impressive, the Belgrade Way (Catalogue of Exhibition). Beograd: Vek Politike; 2004. (Serbian)
15. *Feist PH*. Homelands and Europe: East and Southern European Impressionists. In: *Walther IF*, editor. Impressionism. Köln: Tashen; 2002. p. 530-1.
16. *Ristić V*. Milovanović M: A Memorial Exhibition: Kruševac. Narodni muzej, Umetnička galerija; 1963. (Serbian)
17. *Đuric VJ*. Milan Milovanović-painters and sculptors. Beograd: Prosveta; 1964. (Serbian)
18. *Radulović E*. A War Painter Milan A Milovanović. Kruševac: Narodni muzej; 1996. (Serbian)

Received on October 21, 2010.
Accepted on February 9, 2011.

VOJNOSANITETSKI PREGLED
VOJNOMEDICINSKA AKADEMIJA
Crnotravska 17, 11040 **Beograd, Srbija**
Tel/faks: +381 11 2669689
vsp@vma.mod.gov.rs
vmavsp@hotmail.com

Poziv na reklamiranje u 2011. godini

U prilici smo da vam ponudimo mogućnost oglašavanja i reklamiranja proizvoda i usluga u časopisu „**Vojnosanitetski pregled**“ (VSP). To je sigurno najbolji vid i najzastupljeniji način upoznavanja eventualnih korisnika sa vašim uslugama i proizvodima.

Časopis „**Vojnosanitetski pregled**“, zvanični organ lekara i farmaceuta Vojske Srbije, naučno-stručnog je karaktera i objavljuje radove iz svih oblasti medicine, stomatologije i farmacije. Radove ravnopravno objavljuju stručnjaci iz vojnih i civilnih ustanova i iz inostranstva. Štampa se na srpskom i engleskom jeziku. Časopis izlazi neprekidno od 1944. godine do sada. Jedini je časopis u zemlji koji izlazi mesečno (12 brojeva), na oko 100 strana A4 formata, a povremeno se objavljuju i tematski dodaci (suplementi). Putem razmene ili pretplate VSP se šalje u 23 zemlje sveta. Radove objavljene u VSP-u indeksiraju: *Science Citation Index Expanded (SCIE)*, *Journal Citation Reports/Science Edition*, *Index Medicus (Medline)*, *Excerpta Medica (EMBASE), EBSCO* (preko ove baze VSP je *on line* dostupan od 2002. godine u *pdf* formatu) i *Biomedicina Serbica*.

Cene reklama i oglasa u časopisu „Vojnosanitetski pregled“ u 2009. godini su:

1.	Oglas u crno-beloj tehnici A4 formata za jedan broj	20 000,00 dinara
2.	Oglas u c/b tehnici A4 formata za celu godinu (11-12 brojeva)	200 000,00 dinara
3.	Oglas u boji A4 formata za jedan broj	35 000,00 dinara
4.	Oglas u boji A4 formata za celu godinu (11-12 brojeva)	330 000,00 dinara
5.	Oglas u boji na koricama K3 za jedan broj	50 000,00 dinara
6.	Oglas u boji na koricama K3 za celu godinu (11-12 brojeva)	455 000,00 dinara
7.	Oglas u boji na koricama K2 i K4 za jedan broj	55 000,00 dinara
8.	Oglas u boji na koricama K2 i K4 za celu godinu (11-12 brojeva)	530 000,00 dinara

Za sva objašnjenja, uputstva i ponude zainteresovani kontaktiraju redakciju časopisa „Vojnosanitetski pregled“. Sredstva se uplaćuju na žiro račun kod Uprave javnih plaćanja u Beogradu broj: 840-941621-02 **VMA (za Vojnosanitetski pregled ili za VSP)**, **PIB 102116082**. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om) na adresu: Vojnosanitetski pregled, Crnotravska 17, 11000 Beograd; tel/faks: 011 2669 689, e-mail: vsp@vma.mod.gov.rs ili vmavsp@hotmail.com

UPUTSTVO AUTORIMA

Vojnosanitetski pregled (VSP) objavljuje radove koji ranije nisu nigde publikovani, niti predati za publikovanje redosledom koji određuje uređivački odbor. Uz rad, na posebnom listu, treba dostaviti: izjavu da rad do sada nije objavljen ili prihvaćen za štampu u drugom časopisu; potpise svih koautora; ime, tačnu adresu, brojeve telefona (fiksne, mobilne, faks), *e-mail* adresu, kao i kopiju dokaza o pretplati na časopis za sve autore (i koautore). Za objavljene radove VSP zadržava autorsko pravo. **Od januara 2011. godine primaće se samo radovi napisani na engleskom jeziku.**

Radove slati na adresu: Vojnosanitetski pregled Vojnomedicinska akademija, Institut za naučne informacije, Crnotravska 17, 11040 Beograd.

U VSP-u se objavljuju **uvodnici**, **originalni članci, prethodna** ili **kratka saopštenja**, revijski radovi tipa **opšteg pregleda** (uz uslov da autori navođenjem najmanje 5 autocitata potvrde da su eksperti u oblasti o kojoj pišu), **aktuelne teme** ili **metaanalize**, **kazuistika**, članci iz **istorije medicine**, lični stavovi, naručeni komentari, pisma uredništvu, izveštaji sa naučnih i stručnih skupova, prikazi knjiga, referati iz naučne i stručne literature i drugi prilozi. Radovi tipa originalnih članaka, prethodnih ili kratkih saopštenja, metaanalize i kazuistike objavljuju se uz apstrakte na srpskom i engleskom jeziku.

Rukopis se piše pomoću IBM-PC kompatibilnog računara, sa proredom 1,5. Štampa se na laserskom ili nekom drugom visokokvalitetnom štampaču, uredno i bez ispravki, na čistoj beloj hartiji formata A4, sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize ne smeju prelaziti 16 stranica (sa prilozima); aktuelne teme – osam, kazuistika – šest, prethodna saopštenja – pet, a pisma uredniku, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina.

Radove (tekst i grafičke priloge) treba slati na disketi (CD) i štampane u tri primerka (original i dve kopije). Autori konačnu verziju rukopisa i priloga prihvaćenih za publikovanje predaju u jednom štampanom primerku i na disketi od 3,5" (očišćenoj od „virusa“) ili na kompakt disku. Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003, a samo izuzetno i neki drugi. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

Prispeli radovi kao anonimni podležu uređivačkoj obradi i recenziji najmanje dva urednika/recenzenta. Primedbe i sugestije urednika/recenzenata dostavljaju se autoru radi konačnog oblikovanja. Otisak članka šalje se prvom autoru na korekturu, koji treba vratiti u roku od pet dana. Rukopisi radova prihvaćenih za štampu ne vraćaju se autoru.

Priprema rada

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

1. ***Naslovna strana***

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

b) Ispisuju se puna imena i prezimena autora.

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen i mesta u kojima se ustanove nalaze, sa jasnim obeležavanjem odakle je autor, koristeći standardne znake za fus-note.

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.

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

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

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

Literatura

Literatura se u radu citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i dodaje et al. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma.

Primeri oblika 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 štampaju se sa proredom 1,5 na posebnom listu hartije. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. 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.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se u tri primerka i na disketi (CD). Fotografije treba da budu oštre, na glatkom i sjajnom papiru, do formata dopisnice. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Na svakoj slici treba na poleđini, tankom grafitnom olovkom, označiti broj slike, ime prvog autora i gornji kraj slike. Slike treba obeležiti brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

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

Skraćenice i simboli

Koristiti samo standardne skraćenice, izuzev u naslovu i apstraktu. Pun naziv sa skraćenicom u zagradi treba dati kod prvog pominjanja u tekstu.

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

INSTRUCTIONS TO AUTHORS

Vojnosanitetski pregled (VSP) publishes only previously neither published nor submitted papers in any other journals in the order determined by the Editorial Board. The following should be enclosed with the manuscript: a statement that the paper has not been submitted or accepted for publication elsewhere; a consent signed by all the authors that the paper could be submitted; the name, exact address, phone number, and e-mail address of the first author and co-authors. VSP reserves all copyrights.

Papers should be sent to: Military Medical Academy, Institute for Scientific Information, Crnotravska 17, 11040 Belgrade, Serbia & Montenegro, for "Editorial Board of VSP."

VSP publishes: **editorials, original articles**, **short communications**, **reviews**/**meta-analyses, case reports**, from the **medical history** (general or military), personal views, invited comments, letters to the editor, reports from scientific meetings, book reviews, extensive abstracts of interesting articles from foreign language journals, and other contributions. Original articles, short communications, meta-analyses and case reports are published with abstracts in both English and Serbian.

General review papers type will be accepted by the Editorial Board only if the authors prove themselves as the experts in the fields they write on by citing not less than 5 self-citations.

Papers should be written on IBM-compatible PC, using 12 pt font, and double spacing, and printed on the laser, or other high-quality printer without any corrections, on white bond paper ISO A4 (212×297 mm) with at least 4 cm left margin. **Bold** and *italic* letters should be avoided. Observational and experimental articles, reviews and meta-analyses, should not exceed 16 pages (including tables and illustrations); case reports – 6; short communications – 5; letters to the Editor, reports on scientific meetings and book reviews – 2.

All measurements should be reported in the metric system in terms of the International System of Units (SI). Standard, internationally accepted terms should be used. Papers should be submitted on a diskette (CD) in triplicate (original and two copies).

Authors are requested to submit the final version of manuscript approved for publishing with tables and illustrations on a 3.5" virus free diskette or a CD. **MS Word for Windows** (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs**.** Avoid the use of colors in graphs.

Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the first author for corrections that should be returned within 5 days. Manuscripts accepted for publication are not being returned.

Preparation of manuscript

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

1. ***Title page***

a) The title should be concise but informative. Subheadings should be avoided;

b) full name of each author;

c) name and place of department(s) and institution(s) of affiliation, clearly marked by standard footnote signs.

2. ***Abstract and key words***

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.

3. ***Text***

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.

In the **Introduction** repeat the title of the article, excluding the names of authors. State the purpose of the article and summarize the rationale for the study or observation. Give only strictly pertinent references and do not include data or conclusions from the work being reported.

Methods. Describe your selection of the observational or experimental subjects (patients or experimental animals, including controls) clearly. Identify the methods, apparatus (manufacturer's name and address in parentheses), and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approvement of the Ethnics Committe for the tests in humans and enimals.

Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

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.

References

References should be superscripted and numbered consecutively in the order in which they are first mentioned in the text. **The references must be verified by the author(s) against the original document.** List all authors, but if the number exceeds 6, give 6 followed by et al. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be designated as "in press". Information from manuscripts not yet accepted should be cited in the text as "unpublished observations". References are cited according to the ***International Committee of Medical Journal Editors.*** *Uniform Requirements for Manuscripts Submitted to Biomedical Journals. Ann Intern Med 1997; 126: 36–47. Updated October 2001.*

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

Tables

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

Illustrations

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

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

Abbreviations and symbols

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

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

VOJNOSANITETSKI PREGLED
VOJNOMEDICINSKA AKADEMIJA
Crnotravska 17, 11040 **Beograd**, **Srbija**
Tel/Fax: +381 11 2669689
vmaini1@EUnet.rs
vmavsp@hotmail.com

Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

Godišnja pretplata za 2011. godinu iznosi: 4 000 dinara za građane Srbije, 8 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Sredstva se uplaćuju na tekući račun Vojnomedicinske akademije Beograd kod Uprave za javna plaćanja u Beogradu broj: **840-941621-02 VMA (za Vojnosanitetski pregled ili za VSP)**, **PIB 102116082**. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

PRIJAVA ZA PRETPLATU NA ČASOPIS „VOJNOSANITETSKI PREGLED“

Ime i prezime ili naziv ustanove	
Jedinstveni matični broj građana	
Poreski identifikacioni broj (PIB) za ustanove	
Mesto	
Ulica i broj	
Telefon / telefaks	

Pretplata na časopis „Vojnosanitetski pregled“ (zaokružiti):

1. Lično. Dokaz o pretplati dostavljam uz ovu prijavu.
2. Za pripadnike MO i Vojske Srbije: Dajem saglasnost da se prilikom isplate plata u Računovodstvenom centru MO iz mojih prinadležnosti obustavlja iznos mesečne rate (pretplate).
3. Virmanom po prijemu profakture.

Datum _____	Potpis _____

VOJNOSANITETSKI PREGLED
VOJNOMEDICINSKA AKADEMIJA
Crnotravska 17, 11040 **Beograd**, **Srbija**
Tel/Fax: +381 11 2669689
vmaini1@EUnet.rs
vmavsp@hotmail.com

Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

Godišnja pretplata za 2011. godinu iznosi: 4 000 dinara za građane Srbije, 8 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Sredstva se uplaćuju na tekući račun Vojnomedicinske akademije Beograd kod Uprave za javna plaćanja u Beogradu broj: **840-941621-02 VMA (za Vojnosanitetski pregled ili za VSP)**, **PIB 102116082**. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

PRIJAVA ZA PRETPLATU NA ČASOPIS „VOJNOSANITETSKI PREGLED“

Ime i prezime ili naziv ustanove	
Jedinstveni matični broj građana	
Poreski identifikacioni broj (PIB) za ustanove	
Mesto	
Ulica i broj	
Telefon / telefaks	

Pretplata na časopis „Vojnosanitetski pregled“ (zaokružiti):

1. Lično. Dokaz o pretplati dostavljam uz ovu prijavu.
2. Za pripadnike MO i Vojske Srbije: Dajem saglasnost da se prilikom isplate plata u Računovodstvenom centru MO iz mojih prinadležnosti obustavlja iznos mesečne rate (pretplate).
3. Virmanom po prijemu profakture.

Datum _____	Potpis _____