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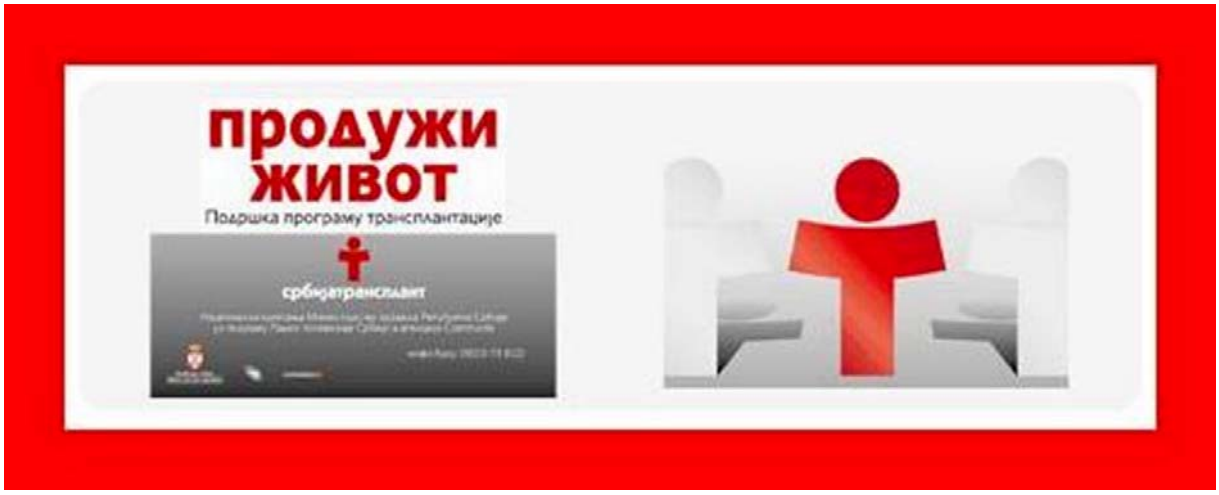

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ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

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Часопис наставља традицију *Војно-санитетског гласника*, који је излазио од 1930. до 1941. године

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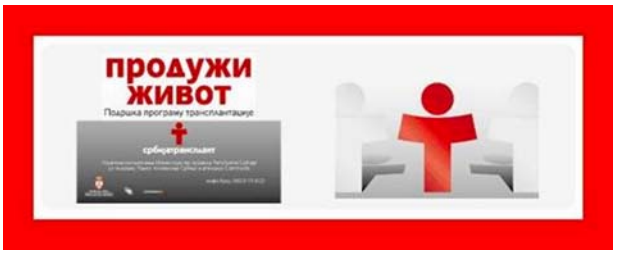

U toku 2010. godine ostvareni su značajni rezultati u okviru nacionalnog programa podrške programu transplantacije organa "Produži život". U tome su se posebno istakli stručnjaci Vojnomedicinske akademije u Beogradu koji su u ovoj godini obavili 17 transplantacija bubrega i 9 transplantacija jetre i zahvaljujući kojima je prikupljeno 23 250 donorskih kartona. U narednoj godini očekuju se još veći uspesi na tom polju.

All the national efforts made in the course of 2010 to support and contribute to the Organ Transplant Program ''Prolong the Life'' produced significant results. An outstanding job was done by the experts from the Military Medical Academy in Belgrade, who during the current year managed to have 23 250 donor cards signed, and perform 17 kidney and 9 liver transplant surgeries. The results to be achieved in the following year are expected to be even greater.

Dragi čitaoci i saradnici,

U nastupajućoj godini naš i vaš *Vojnosanitetski pregled* treba da dobije svoj prvi impakt faktor. U očekivanju tog zajedničkog uspeha, uz zahvalnost na dosadašnjoj, veoma plodnoj saradnji, u ime Uređivačkog odbora, Redakcije i Izdavača časopisa, kao i u svoje lično ime, želim vam ispunjenje svih želja, ličnu sreću, profesionalno zadovoljstvo i mnogo, mnogo radosnih trenutaka u novoj 2011. godini.

Ž I V E L I ! ! !

Srdačno,
Vaša prof. dr Silva Dobrić
glavni i odgovorni urednik VSP

Dear Readers and Colleagues,

I would like to inform you that our and your *Vojnosanitetski pregled* has been nominated for awarding its first impact factor. Looking forward to that event, and expressing our deep and sencere gratitude for all efforts you have made with the aim of further developing and enhancing our already successful cooperation, I wish you, on behalf of the Editorial Board members, the Editorial Staff, the Publisher, and myself a lot of happiness in your life, professional success, fulfilment of all your wishes and many joyful moments in the coming year of 2011!

MARRY CHRISTMAS AND HAPPY NEW YEAR!

Best regards,
Prof. Silva Dobrić
Editor-in-Chief

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Histohemijska i imunohistohemijska analiza rupturisanog zida aneurizme aterosklerotične abdominalne aorte

Histochemical and immunohistochemical analysis of ruptured atherosclerotic abdominal aortic aneurysm wall

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Apstrakt

Uvod/Cilj. Najznačajnija komplikacija aneurizme aterosklerotične abdominalne aorte (AAA) je njena ruptura koja najčešće počinje rascepom intime i rupturom plaka. Cilj ove studije bio je utvrđivanje imunohistohemijskih i morfofunkcijskih karakteristika ćelija u sastavu zida aterosklerotične aneurizme abdominalne aorte sa rupturom. **Metode.** Korišćeno je 20 uzoraka aterosklerotičnih AAA sa rupturom zida, dobijenih tokom autopsija. Uzorci su fiksirani u 4% formalinu i kalupljeni u paraplastu. Rezovi debljine 5 μm bojeni su histohemijski (*Heidenhain azan* i *Periodic acid Schiff* – PAS) i imunohistohemijskom tehnikom DAKO LSAB+/HRP koja je primenjena za identifikaciju sledećih antigena: α-glatkomišićni aktin (α-SMA), vimentin, teški lanci glatkomišićnog miozina (MHC), dezmin, S-100 protein, CD45 i CD68 (DAKO specifikacija). **Rezultati.** Aterosklerotičnu AAA sa rupturom zida karakteriše potpuno odsustvo endotelnih ćelija, disrupcija bazalne membrane i unutrašnje elastične lamine, kao i prisustvo ostataka ekstenzivnih komplikovanih izrazito hipocelularnih plakova u intimi. Na marginama plakova i u mediji bile su prisutne glatke mišićne ćelije imunoreaktivne na α-SMA i vimentin, leukocitna infiltracija i veliki broj penastih ćelija. Jedan broj penastih ćelija pokazao je imunoreaktivnost na CD68, dok su druge pokazale imunoreaktivnost na vimentin i S-100 protein. Medija je bila istanjena sa dezorganizovanim elastičnim lamelama, dok je u adventiciji bila prisutna izrazita leukocitna infiltracija, što ukazuje na zapaljenjski proces. U rupturisanoj intimi aterosklerotičnih AAA nađeni su ostaci komplikovane aterosklerotične lezije klasifikovane kao tip VI. **Zaključak.** Ruptura aneurizme nastaje iz primarnog rascepa intime, koji se širi u istanjenu mediju i adventiciju. Rupturu uzrokuju nestabilni aterom, hipocelularnost, gubitak kontraktilnih karakteristika glatkih mišićnih ćelija u intimi i mediji, neovaskularizacija medije, kao i aktivnost makrofaga u leziji.

Ključne reči:
aorta, abdominalna, aneurizma; ruptura; arterioskleroza; imunohistohemija; histologija.

Abstract

Background/Aim. The main complication of the atherosclerotic abdominal aortic aneurism (AAA) is her rupture that begins with lesion in intima and rupture. The purpose of this work was to determine immunocytochemical and morphofunctional characteristics of the cells in aortic wall in ruptured atherosclerotic abdominal aortic aneurysm. **Method.** During the course of this study, 20 samples of atherosclerotic AAA were analyzed, all of them obtained during authopsy. The samples were fixed in 4% formalin and embedded in paraffin. Sections of 5 μm thickness were stained histochemically (of Heidenhain azan stain and Periodic acid Schiff – PAS stain) and immunocytochemically using a DAKO LSAB+/HRP technique to identify α-smooth muscle actin (α-SMA), vimentin, myosin

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heavy chains (MHC), desmin, S-100 protein, CD45 and CD68 (DAKO specification). **Results.** The results of our study showed that ruptured atherosclerotic AAA is characterized by a complete absence of endothelial cells, the disruption of basal membrane and internal elastic lamina, as well as a presence of the remains of hypocellular complicated atherosclerotic lesion in intima. On the plaque margins, as well as in the media, smooth muscle cells (SMCs) are present, which express a α-SMA and vimentin (but without MHC or desmin expression), as well as leukocyte infiltration, and a large number of foam cells. Some of the foam cells show a CD68- immunoreactivity, while the others show vimentin- and S-100 protein-immunoreactivity. Media is thinned out with a disorganized elastic lamellas, while adventitia is characterized by inflammatory inflitrate (infection). **Conclusion.** Rupture of aneurysm occurs from the primary intimal disruption, which spreads into thinned out media and adventitia. Rupture is caused by unstable atherom, hypocellularity, loss of contractile characteristics of smooth muscle cells in intima and media, neovascularization of the media, as well as by the activity of the macrophages in the lesion.

Key words:
aortic aneurysm, abdominal; rupture; arteriosclerosis; immunohistochemistry; histology.

Uvod

Aterosklerotične aneurizme abdominalne aorte (AAA) nastaju usled prisustva uznapredovalih aterosklerotičnih lezija tipa IV (aterom) ili V (fibroaterom) u zidu aorte [1, 2]. Uznapredovali aterosklerotični plak uzrokuje dilataciju dela zida aorte i često podleže oštećenjima poput disrupcije plaka, hemoragije ili tromboze, što dovodi do nastanka komplikovane aterosklerotične lezije (tipa VI). Prema vrsti oštećenja na površini plaka, kod aterosklerotične AAA mogu da se nađu lezije tipa VIa (plak sa rupturom), VIb (plak sa hemoragijom) ili VIc (plak sa trombozom). Kod aterosklerotične AAA sa rupturom zida u plaku istovremeno mogu da budu prisutne i sve tri vrste komplikacija, što predstavlja leziju tipa VIa, b, c [2].

Najznačajnija komplikacija aterosklerotične AAA je njena ruptura, koja najčešće počinje primarnim rascepom intime (rupturom plaka) koji se zatim širi u dublje slojeve aortnog zida [3, 4]. Povećana aktivnost proteolitičkih enzima u leziji i posledična degradacija ekstracelularnog matriksa, promovišu rupturu aneurizme [4]. Sa aspekta citohistoloških svojstava aortnog zida aterosklerotične AAA koji doprinose rupturi aneurizme, kao najznačajniji činioci koji promovišu rupturu izdvajaju se strukturna slabost zida koja je uslovljena sastavom lezije [5], prisustvo makrofaga, penastih ćelija poreklom od makrofaga i proteolitičkih enzima [6], prisustvo inflamatornih ćelija u leziji [7], kao i izloženost zida povećanom hemodinamičkom stresu [8].

U savremenoj literaturi još uvek nisu sasvim razjašnjeni svi faktori koji dovode do nastanka aneurizme, a zatim i do njene rupture, posebno sa aspekta detaljne morfofunkcionalne analize. Veliki broj autora koji se bave istraživanjem aterosklerotične AAA, slaže se u tome da bi razumevanje procesa koji su zastupljeni u razvoju ove lezije i mehanizama njihove regulacije, kao i precizno definisanje fenotipa ćelijskih populacija koja učestvuju u tim procesima, doprinelo razvoju novih terapijskih strategija u lečenju aterosklerotične AAA [3]. Zbog toga je cilj ove studije bio utvrđivanje imunohistohemijskih karakteristika fenotipski heterogenih ćelija u rupturisanom aterosklerotičnom plaku abdominalne aortne aneurizme, odnosno utvrđivanje njihovih morfofunkcionalnih karakteristika, kao i njihove potencijalne uloge u rupturi zida aterosklerotične AAA.

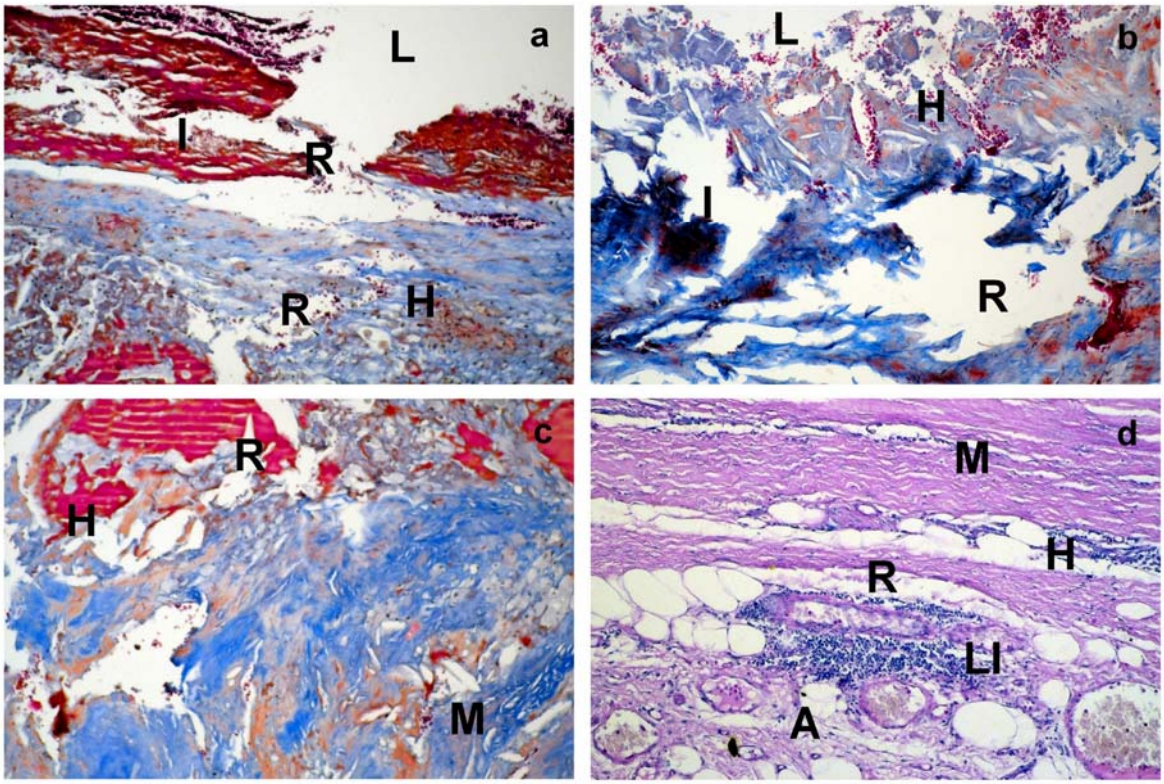
Metode

Analizirano je 20 uzoraka aterosklerotičnih aneurizmi abdominalne aorte sa rupturom zida dobijenih tokom autopsija osoba oba pola, umrlih od nevaskularnih i vaskularnih uzroka, urađenih na Odeljenju patologije Medicinskog fakulteta Univerziteta u Kragujevcu. Za analizu morfoloških promena u zidu aorte i sastava ekstracelularnog matriksa primenjena su selektivna histohemijska bojenja (*Heidenhain azan i Periodic acid Schiff* – PAS), prema standardnom protokolu [9]. Za potrebe imunohistohemijskog bojenja, tkivo je fiksirano u 4% neutralnom puferovanom formaldehidu 24 časa i kalupljeno u paraplastu. Rezovi debljine 5 μm, montirani su na posebne visokoadherentne pločice *SuperFrost* i sušeni na temperaturi od 56 ºC u toku 1 sata. Procedura imunohistohemijskog bojenja podrazumevala je postupke demaskiranja antigena, blokiranja endogene peroksidaze, inkubiranja preparata sa primarnim antiserumom i postupak izvođenja imunohistohemijske metode – LSAB+/HRP, na način koji smo prethodno opisali [10, 11]. Tokom imunohistohemijskog bojenja korišćeni su sledeći primarni antiserumi u datim razblaženjima: vimentin (1:100), α-glatkomišićni aktin (α-*Smooth Muscle Actin* – α-SMA) (1:25), teški lanci glatkomišićnog miozina (*Myosin Haevy Chains* – MHC) (1:50), dezmin (1:10), S-100 protein (1:200), CD45 (zajednički antigen leukocita – *Leucocyte Common Antigen* – LCA) (1:50) i CD68 (1:50).

Rezultati

Analizom rezultata dobijenih primenom histohemijskih tehnika, zapaženo je da su aterosklerotične promene na svim uzorcima zahvatale ceo aortni zid. U intimi je zapaženo prisustvo ostataka ekstenzivne aterosklerotične lezije, medija ispod plaka bila je atrofično istanjena, dok je u adventiciji prisutan hronični zapaljenjski infiltrat. Usled rupture aneurizme, bila su prisutna izrazita oštećenja intime, medije i adventicije, i posledična hemoragija u svim slojevima zida (slika 1 a–d).

Na svim analiziranim uzorcima, endotel i bazalna membrana u potpunosti su nedostajali. U subendotelu intime nalazili su se ostaci komplikovanog hipocelularnog aterosklerotičnog plaka koji je zauzimao veoma široko područje i



Sl. 1 – Histohemijska analiza rupturisane aterosklerotične aneurizme abdominalne aorte
a, b i c – ruptura (R) intime (I) i medije (M). Zapaža se hemoragija (H) u lumenu (L) i delovima zida (*Heidenhain azan* bojenje, ×64); d – istanjena medija (M) sa rupturom *vasa vasorum* (R) i posledičnom hemoragijom (H); leukocitni infiltrat (LI) u adventiciji (A) (*Periodic acid Schiff* – PAS bojenje, ×64)

bio sastavljen iz većeg broja manjih plakova. Na mestima na kojima su postojali manji plakovi, mogla je da se uoči rekanalizacija, odnosno novoformirani lumen, ruptura *vasa vasorum* i posledična hemoragija u celom subendotelu. Na fibroznoj kapi nekih uzoraka, zapažala se ruptura i ulceracija sa parijetalnim trombom (slika 1 a, b). Unutar plaka, bili su prisutni holesterolski kristali i ćelijski detruitus. Na obodima plaka, zapažale su se intenzivna leukocitna infiltracija, glatke mišićne ćelije i penaste ćelije.

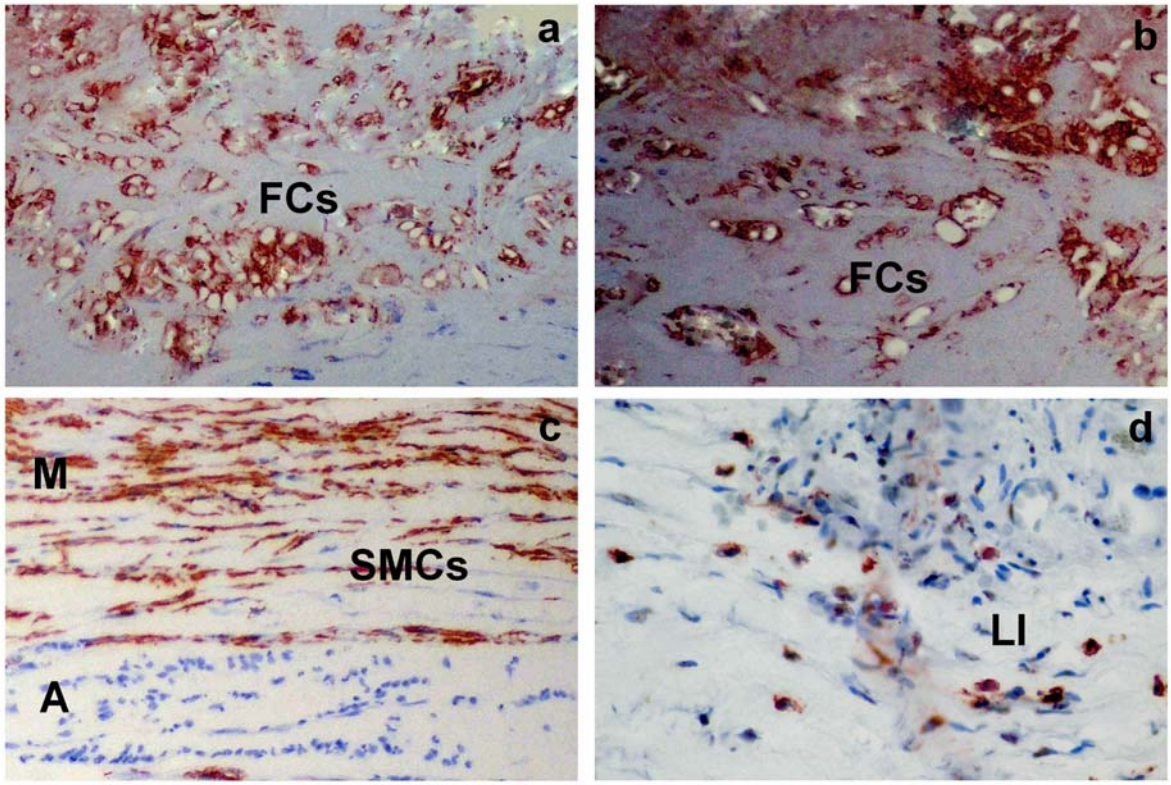
Unutrašnja elastična membrana bila je prekinuta rupturom, a u ostalim delovima zida u potpunosti je nedostajala. Na mestima na kojima je bila prisutna, duplicirana je, a prostor između te dve membrane popunjavalo je fibrozno vezivo, sastavljeno od debelih kolagenih vlakana. U istanjenoj tunici mediji zapažene su ispravljene, fokalno duplicirane, a na pojedinim mestima prekinute elastične lamele, dosta kolagenih vlakana i mala količina osnovne supstance. Na svim analiziriranim uzorcima, u istanjenoj mediji, krv disekcijom (iz primarne rupture intime) stvarala je lažni lumen, čiji spoljašnji zid (deo medije i adventicija) je rupturirao, takođe. Osim toga, svuda u mediji bili su prisutni i novoformirani krvni sudovi, koji su, takođe, podlegali rupturi. Spoljašnja elastična membrana bila je sasvim tanka i teško se zapažala zbog potpune fibroze i rupture medije (slika 1 a–d).

Adventicija je bila sastavljena iz rastresitog vezivnog tkiva sa dosta kolagenih i malo elastičnih vlakana, fibroblasta, limfocita i histiocita. Adventicijalni krvni sudovi (*vasa vasorum*) bili su zadebljalih zidova, a svuda u adventiciji, bila je prisutna izrazita leukocitna infiltracija (slika 1d).

Na marginama rupturisanih plakova, zapažena je prisustvo heterogene ćelijske populacije i ćelijskog detruitusa, dok su ostaci lipidnog jezgra na svim uzorcima bili acelularni i ispunjeni lipidnim kristalima. Ispitivanjem distribucije antigena specifičnih za glatke mišićne ćelije zapaženo je da su u sastavu ove ćelijske populacije na marginama plakova, kao i u mediji bile prisutne glatke mišićne ćelije koje su pokazivale imunoreaktivnost na α-SMA i vimentin, dok je reakcija na MHC i dezmin izostala. Ispitivanjem distribucije S-100 proteina, utvrđeno je njegovo prisustvo u ćelijama na granici između intime i medije.

U ćelijskoj populaciji na margini plakova utvrđeno je i prisustvo penastih ćelija različitih karakteristika. Jedan broj penastih ćelija pokazivale su CD68-imunoreaktivnost, a druge vimentin-imunoreaktivnost. Vimentin-imunoreaktivne penaste ćelije bile su lokalizovane pretežno u dubljim oblastima intime na njenoj granici sa medijom. U dubljim oblastima intime, zapažalo se i prisustvo penastih ćelija imunoreaktivnih na S-100 protein. Svuda u adventiciji bila je prisutna izrazita leukocitna infiltracija (slika 2 a– d).

Ispitivanjem distribucije CD45 antigena utvrđeno je da se najveći broj ćelija koje eksprimiraju ovaj antigen nalazio na obodu plaka, znatno manji broj na samom mestu rupture, dok je najveći broj bio prisutan u spoljašnjem delu medije i adventicije.



Sl. 2 – Imunohistohemijska analiza ćelijske populacije rupturisane aterosklerotične aneurizme abdominalne aorte,
a – penaste ćelije (FCs) poreklom od monocitno/makrofagne linije (imunohistohemijsko bojenje na CD68, ×256); b – penaste ćelije (FCs) poreklom od glatkih mišićnih ćelija (imunohistohemijsko bojenje na vimentin, ×256); c – glatke mišićne ćelije (SMCs) sintetskog fenotipa u istanjenoj mediji (M) i na granici sa adventicijom (A) (imunohistohemijsko bojenje na vimentin, ×128) i d – leukocitna infiltracija (LI) u adventiciji (imunohistohemijsko bojenje na CD45, ×128).

Diskusija

Prema rezultatima dobijenim u ovoj studiji, aterosklerotičnu AAA sa rupturom zida karakteriše prisustvo ostataka rupturisanog aterosklerotičnog plaka sa hemoragijom i razvijenim trombnim masama. U sastavu plaka, zapažaju se ostaci manjih, sekundarnih plakova međusobno razdvojenih rekanalisanim lumenima kroz koje se širi disekcija zida i koji predstavljaju predilekciono mesto rupture. Na svim analiziranim uzorcima u ovoj studiji, na samom mestu rupture, u sastavu najprominentnijeg plaka, bilo je prisutno ekstenzivno acelularno lipidno jezgro, dok je na njegovom spoljašnjem, fibrozno izmenjenom obodu, u svim uzorcima bila prisutna heterogena ćelijska populacija. Evidentno je da je komplikovani plak aterosklerotične AAA nastao od primarnog ateroma (lezije tipa IV), komplikovanog disrupcijom površine plaka, hemoragijom i trombozom, što je u saglasnosti sa dostupnim literaturnim podacima [1].

Poređenjem rezultata dobijenih u ovoj studiji sa podacima iz literature o uzrocima rupture plaka [4, 5], moglo bi da se pretpostavi da je jedan od uzroka rupture plaka, a zatim i same aneurizme, mogao da bude sastav aterosklerotične lezije, odnosno strukturna slabost zida uzrokovana prisustvom nestabilnog ateroma. Naime, prema podacima iz dostupne literature, lezije tipa IV (ateromi), posebno su podložne rupturi [5]. Dokazano je da su lezije u kojima dominira veliko lipidno jezgro (ateromi), nestabilne i da lako podležu rupturi i komplikacijama plaka, zato što, za razliku od stabilnijih fibroateroma (lezija tipa V) ne poseduju debelu fibroznu kapu koja doprinosi očuvanju njihove strukture [1].

Analizom populacije glatkih mišićnih ćelija na obodima uzoraka plakova u našoj studiji, utvrđeno je da je ovaj fenotip prisutan u leziji (imunoreaktivnost na α-SMA), ali da ćelije ne poseduju kontraktilne karakteristike. Imunohistohemijskim bojenjima utvrđeno je da populacija glatkih mišićnih ćelija osim α-SMA eksprimira i vimentin, dok reakcija na dezmin izostaje. Pored toga, i u mediji uzoraka analiziranih u ovoj studiji, takođe, uočen je potpuni izostanak dezminske ekspresije uz prisustvo vimentin-imunoreaktivnih ćelija, što sugeriše da medijalne glatke mišićne ćelije, takođe, eksprimiraju samo α-SMA i vimentin. Prema rezultatima naših prethodnih studija [8, 10, 11], kao i studija drugih autora [12–14], gubitak ekspresije dezmina, kao markera visokodiferenciranog kontraktilnog fenotipa, uz istovremenu ekspresiju vimentina, prvi je znak u procesu modifikacije kontraktilnog u sintetski fenotip. Pošto se ova dva intermedijarna filamenta koeksprimiraju u vaskularnim glatkim mišićnim ćelijama, „pomeranje“ ekspresije ka vimentinu svedoči o gubitku kontraktilnih karakteristika glatkih mišićnih ćelija, odnosno, o njihovoj modifikaciji u sintetski fenotip [10, 11, 13]. Ovako izmenjene, sintetski aktivne glatke mišićne ćelije u plaku aterosklerotične aneurizme, prema rezultatima naših prethodnih istraživanja, kao i istraživanja drugih autora, potiču kako od matične populacije intimalnih glatkih mišićnih ćelija, tako i od ćelija koje migriraju iz medije u intimu i doprinose formiranju plaka u ranim fazama ateroskleroze [8, 15, 16]. Osim gubit-

ka kontraktilnih svojstava glatkih mišićnih ćelija, rezultati histohemijskih i imunohistohemijskih bojenja pokazali su da su ostaci tankih fibroznih delova na marginama plakova hipocelularni, dok su ostaci lipidnih jezgara u potpunosti acelularni, što dodatno doprinosi nestabilnosti lezije i rupturi plaka. Naime, prema podacima iz literature, prisustvo glatkih mišićnih ćelija na obodima plaka i njihova morfofunkcionalna očuvanost doprinose stabilnosti lezije [12].

Osim glatkih mišićnih ćelija sintetskog fenotipa, rezultati su pokazali da je u sastavu ćelijske populacije na margini plaka prisutan i jedan broj glatkih mišićnih ćelija koje pokazuju imunoreaktivnost na α-SMA, vimentin i S-100 protein uz prisutne lipidne kapi u citoplazmi, pa odaju utisak penastih ćelija. Nalaz vimentin-imunoreaktivnih penastih ćelija (koji ukazuje na njihovo glatkomišićno poreklo) u saglasnosti je sa našim prethodnim rezultatima [11], kao i sa rezultatima drugih autora, po kojima jedan broj glatkih mišićnih ćelija eksprimira *scavenger* receptore i kompetitivno učestvuje sa makrofagima u akumulaciji lipida i stvaranju penastih ćelija, što se dovodi u vezu sa neuroektodermalnim poreklom glatkih mišićnih ćelija u zidu aorte [16, 17]. Zbog toga što potiču od nervnog grebena, ove ćelije eksprimiraju S-100 protein, a kako eksprimiraju sintetski fenotip (imunoreaktivnost na vimentin), karakteriše ih i proliferativna aktivnost [11, 13]. Na svim analiziranim uzorcima u ovoj studiji, zapažena je ekspresija S-100 proteina na granici intime i medije. Prema literaturnim podacima S-100 protein eksprimira se u ćelijama neuroektodermnog porekla koje se nalaze u procesima diferencijacije, proteinske fosforilacije i proliferacije, kojima posreduje Ca^{++} [18]. Ekspresija ovog antigena karakteristična je, između ostalih ćelijskih tipova, i za vaskularne glatke mišićne ćelije, kao i za vaskularne dendritske ćelije [19, 20]. Uz to, u novijim istraživanjima dokazano je da heterokompleks sastavljen od S-100A8 i S-100A9 kalcijum-vezujućih proteina (*calprotectin*), pokazuje visok afinitet za vezivanje masnih kiselina [21]. Moguće je dakle, da glatke mišićne ćelije koje zbog svog neuroektodermnog porekla i proliferativne aktivnosti eksprimiraju S-100 protein, upravo zahvaljujući ekspresiji ovog antigena poseduju sposobnost za akumulaciju lipida i modifikaciju u penaste ćelije.

Kao što je prethodno pomenuto, ekspresija S-100 proteina karakteristična je i za vaskularne dendritske ćelije [19, 20]. Međutim, u uzorcima analiziranim u ovoj studiji, usled masivne nekroze i prisutnog ćelijskog detruitusa, na nivou svetlosne mikroskopije nije moguće potvrditi precizne citomorfološke karakteristike vaskularnih dendritskih ćelija. Sigurno je da jedan broj ćelija imunoreaktivnih na S-100 protein čine i vaskularne dendritske ćelije, jer je prema dostupnim podacima iz literature poznato da su ove antigen-prezentujuće ćelije, zastupljene u manjoj meri u normalnoj intimi, dok se u neuporedivo većem broju nalaze u aterosklerotičnim lezijama velikih arterija, da ne akumuliraju lipide, kao i da formiraju kontakte sa limfocitima i učestvuju u razvoju inflamatornih procesa [19, 20].

Na rupturu aneurizme, prema prethodno pomenutim podacima iz literature, osim sastava plaka i morfofunkcionalnih karakteristika glatkih mišićnih ćelija, utiče i prisustvo makrofaga i njihova funkcionalna aktivnost u leziji [4–6], kao i prisustvo zapaljenjskog infiltrata [7]. Rezultati ove studije pokazali su da se pored penastih ćelija koje potiču od glatkih mišićnih ćelija, u ćelijskoj populaciji na margini plaka nalaze i CD68-imunoreaktivne penaste ćelije, što sugeriše da ove ćelije potiču od monocitno-makrofagne loze [22]. Prema podacima iz literature, u inicijalnim fazama lezije (u fazi aktivacije endotela i stadijumu masne trake) monociti, odnosno makrofagi predstavljaju glavne prekursore penastih ćelija. Od stadijuma preateroma, najveći deo penastih ćelija nastaje od glatkih mišićnih ćelija [11]. Penaste ćelije u ranim fazama ateroskleroze akumuliraju lipide intracelularno, ali već od stadijuma preateroma količina lipida prevazilazi kapacitete penastih ćelija koje, pri tom, podležu nekrozi i oslobađanju svog sadržaja u ekstracelularni prostor, što uzrokuje stvaranje velike lipidne mase u plaku, koja se u sledećem stadijumu lezije organizuje kao aterom [11]. Na taj način makrofagi direktno utiču na stvaranje nestabilnog ateroma u intimi.

Osim toga, makrofagi sintetišu matriksne metaloproteinaze (MMP) koje razgrađuju komponente ekstracelularnog matriksa intime i medije i utiču na ćelijsku proliferaciju, migraciju, diferencijaciju, angiogenezu, apoptozu i aktivaciju hemokina, čime direktno i posredno doprinose slabljenju vaskularnog zida i promovišu rupturu aneurizme [6]. Utvrđeno je da je za rupturu aneurizme od najvećeg značaja prisustvo MMP1, MMP8 i MMP9 u leziji, kao i da je njihova koncentracija u aneurizmi sa rupturom značajno veća u odnosu na aneurizmu bez rupture zida [23, 24]. Dokazano je da je koncentracija ovih MMP najveća na mestima rupture aneurizme, da je njihova funkcija degradacija kolagenih vlakana (kolagenaze), kao i da ih pored makrofaga, sintetišu i endotelne ćelije kao i glatke mišićne ćelije [6]. Proteolitičkom aktivnošću ovih enzima i razlaganjem kolagenih vlakana zid podleže hemodinamičkom pritisku i rupturira. Osim toga, dokazano je da MMP2 i MMP9 uzrokuju regulaciju nagore genske ekspresije citokina angiogeneze čime doprinose neovaskularizaciji aterosklerotične AAA, a time i njenoj rupturi [3, 25]. Izražena neovaskularizacija bila je prisutna na svim uzorcima analiziranim u ovoj studiji. Novoformirani krvni sudovi na analiziranim uzorcima, predstavljali su mesta širenja rupture, jer su njihovi rupturirani zidovi bili u kontinuitetu sa primarnom rupturom intime. Na taj način, hemoragija iz primarnog intimalnog procepa, širila se putem novoformiranih krvnih sudova u dublje delove zida i uzrokovala procepe u mediji, što je bilo prisutno u svim analiziranim uzorcima.

Ispitivanjem distribucije CD45 antigena, utvrđeno je da je u svim analiziranim uzorcima u svim delovima zida bila prisutna leukocitna infiltracija. Najveći broj leukocita (CD45-imunoreaktivnih ćelija) bio je prisutan na marginama plaka, spoljašnjem delu medije i adventiciji, dok je na samom mestu rupture, broj leukocita bio znatno manji. Ovakav rezultat u saglasnosti je sa rezultatima novijih studija o uticaju leukocitnog infiltrata na rupturu aneurizme. Naime, uprkos nekadašnjem mišljenju da prisustvo inflamatornih ćelija u leziji doprinosi rupturi plaka, a zatim i zida aterosklerotične AAA [7], novija istraživanja pokazala su da je broj infiltriranih CD45 imunoreaktivnih ćelija manji na samom mestu rupture, nego u ostalim delovima zida aterosklerotične AAA [23]. Ovakvi rezultati, iako deluju kontradiktorno, sugeri-

šu da inflamacija tkiva ima mali uticaj na proces rupture aneurizme. Međutim, još uvek nije sasvim poznato na koje načine inflamatorna reakcija utiče na rupturu aterosklerotične AAA, ali se za sada zna da u kontroli i regulaciji inflamatornih procesa značajnu ulogu imaju MMP koje deluju na modifikaciju proinflamatornih citokina, hemokina i membranskih receptora [3].

Zaključak

Rezultati analize histohemijskih i imunohistohemijskih karakteristika aterosklerotične AAA, kao i morfofunkcionalnih karakteristika ćelija u njenom sastavu, sugerišu da se kao najznačajniji faktori koji doprinose rupturi aneurizme mogu izdvojiti: prisustvo nestabilnog, komplikovanog aterosklerotičnog plaka u intimi, prisustvo makrofaga i glatkih mišićnih ćelija sintetskog fenotipa koji sintetišu MMP, hipocelularnost, gubitak elastičnih svojstava medije i kontraktilnih karakteristika njenih glatkih mišićnih ćelija i prisustvo novoformiranih krvnih sudova u mediji, dok manji uticaj na rupturu ima leukocitna infiltracija. Usled opisanih degenerativnih promena zid aterosklerotične AAA gubi sposobnost da se kompenzatornom dilatacijom prilagodi uslovima povišenog pritiska i podleže rupturi.

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Značaj koncentracije rezistina kod metabolički uslovljenih bolesti

The significance of resistin concentration in metabolical diseases

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Apstrakt

Uvod/Cilj. Masno tkivo sekretuje brojne hormonski aktivne supstance koje imaju važnu ulogu u razvoju različitih metaboličkih oboljenja. Cilj ove studije bio je da se utvrdi značaj rezistina kod bolesnika sa različitim formama metaboličkih bolesti. **Metode.** Istraživanje je sprovedeno u bolnici u Lapljem Selu i obuhvatilo je 102 bolesnika koji boluju od *diabetes mellitus*-a, kardiovaskularnih bolesti i bubrežne insuficijencije, najmanje trećeg stepena. Svim ispitanicima obavljena su antropometrijska merenja, kontrolisane osnovne hematološke i biohemijske analize i koncentracija rezistina. **Rezultati.** Prosečna starost ispitanika, 69 (67,6%) muškaraca i 33 (32,4%) žena, bila je 55 ± 9,3 godine. Dve trećine naših bolesnika bili su pušači. Srednja vrednost sistolnog arterijskog krvnog pritiska bila je 165 ± 17,1 mmHg, a dijastolnog 100 ± 9,23 mmHg, indeks telesne mase bio je u proseku 28,5 ± 3,4 kg/m^2, obim kukova 100 ± 10,8 cm, obim struka 110 ± 17,4 cm, a odnos obim kukova/obim struka 1,05 ± 0,14. Više od jedne trećine ispitanika imalo je neku formu kardiovaskularnih bolesti, bubrežnu insuficijenciju imalo je skoro petina bolesnika, dok je *diabetes mellitus* bio verifikovan kod 44,1% ispitivane populacije. Značajno veće vrednosti koncentracije rezistina utvrđene su kod bolesnika sa *diabetes mellitus*-om i bubrežnom insuficijencijom. U odnosu na nivo rezistina bolesnici su se, prema broju metaboličkih oboljenja po jednom bolesniku, statistički značajno razlikovali ($F = 11,80$; $DF = 4,61$; $p < 0,001$). Spearmanovom korelacijom ($r = 0,66$; $p < 0,001$) utvrđena je statistički značajna povezanost između broja bolesti kod jednog ispitanika i nivoa rezistina. **Zaključak.** Veći broj manifestnih bolesti po jednom ispitaniku, *diabetes mellitus* i bubrežnu insuficijenciju, karakterišu visoke vrednosti rezistina.

Ključne reči:
metaboličke bolesti; masno tkivo; rezistin; osetljivost i specifičnost.

Abstract

Background/Aim. Adipose tissue secrets a number of hormonal active substances that play an important role in the development of various metabolic diseases. The aim of this study was to determine the resistin significance in patients with various forms of metabolic diseases. **Methods.** The survey was conducted in a hospital in Laplje Selo, and included 102 patients suffering from *diabetes mellitus*, cardiovascular disease and renal insufficiency. All patients were verified and anthropometric characteristics and the basic hematological and biochemical analysis and resistin level were controlled. **Results.** The average age of the patients, 69 (67.6%) men and 33 (32.4%) women, was 55 ± 9.3 years. It was about two-thirds of smokers. The values of mean arterial blood pressure suggested that our patients had expressed hypertension, body mass index (28.5 ± 3.4 kg/m^2) pointed to excessive well-fed, the circumference of hips (100 ± 10.8 cm), waist circumference (110 ± 17.4 cm) and the ratio of hip/waist circumference (1.05 ± 0.14) indicated a significant intra-abdominal distribution of fat tissue. More than one third of our patients had a certain form of cardiovascular diseases, renal failure was present almost in a fifth of patients, while *diabetes mellitus* was verified in 44.1% of the studied population. Significantly higher values of resistin concentrations were determined in patients with renal insufficiency and *diabetes mellitus*. In relation to the level of resistin, according to the number of metabolic diseases per patient, a statistically significant difference was achieved ($F = 11.80$, $DF = 4.61$, $p < 0.001$). Spearman correlation ($r = 0.66$, $p < 0.001$) found a statistically significant correlation between the number of diseases in one subject and the levels of resistin. **Conclusion.** In patients with *diabetes mellitus* and renal failure higher concentrations of resistin were recorded.

Key words:
metabolic diseases; adipose tissue; resistin; sensitivity and specificity.

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Uvod

Tradicionalna predstava o masnom tkivu kao pasivnom rezervoaru skladištenja viška energije od 1987. godine nije više aktuelna. Tada je, naime, utvrđeno da je masno tkivo jedan od glavnih endokrinih organa za sintezu brojnih adipokina koji regulišu gojaznost kod glodara [1, 2]. Do danas je otkriveno pedesetak produkata masnog tkiva, ali se kao potencijalni izvor adipokina navode i makrofagi [3, 4]. Rezistin je protein bogat cisteinom, sastavljen od 108 aminokiselina molekulske težine 12,5 kDa. Sekretuje se u adipocitima i ima uticaja na razvoj insulinske rezistencije, proporcionalno stepenu gojaznosti [1, 5].

Gojaznost je udružena sa brojnim komorbidnim stanjima koja uslovljavaju povećan kardiovaskularni morbiditet i *diabetes mellitus* (DM) tipa 2. Pored toga, postoji pozitivna korelacija sa faktorima rizika kao što su povišen nivo triglicerida, hipertenzija, glikozna intolerancija [6, 7]. Rezistin je u povećanoj koncentraciji povezan sa većom incidencijom insulinske rezistencije, međutim, svoj uticaj na srčanu slabost pokazuje mehanizmom koji je nezavistan od insulinske rezistencije [8]. Ipak, uloga različitih koncentracija rezistina na pojavu ateroskleroze, nezavisno od uobičajenih faktora rizika, ostaje da se utvrdi [9, 10].

Cilj našeg istraživanja bio je da se utvrdi značaj rezistina kao markera metabolički uslovljenih bolesti.

Metode

Istraživanje je sprovedeno u bolnici Laplje Selo, KBC-Priština sa sedištem u Gračanici (Kosovo i Metohija) u toku 2008. godine. U studiju su bila uključena 102 ispitanika kod kojih su postojali kliničko-laboratorijski znaci dijabetesa melitusa, kardiovaskularnih oboljenja (KVO) i bubrežne slabosti (BI), najmanje trećeg stepena, koja ne zahteva dijaliznu depuraciju.

Studiju je odobrio Etički komitet Medicinskog fakulteta Priština/Kosovska Mitrovica. Svi ispitanici pismeno su potvrdili svoj dobrovoljni pristanak za učešće u ispitivanju.

Svim ispitanicima određivane su antropometrijske karakteristike: telesna masa (kg), telesna visina (cm), indeks telesne mase (ITM) izračunavan formulom: količnik između telesne mase izražene u kg i kvadrata telesne visine izražene u m^2, obim struka (cm), obim kukova (cm) i izračunavan je njihov količnik – WHR (*Waist to Hip Ratio*).

Svim ispitanicima utvrđen je broj leukocita i eritrocita, koncentracija hemoglobina, glukoze, ureje, kreatinina, holesterola, LDL-holesterola, triglicerida, ukupnih proteina, albumina i rezistina u krvi. Koncentracija glukoze, ureje, kreatinina, holesterola, LDL-holesterola, triglicerida, ukupnih proteina i albumina određena je spektrofotometrijskom metodom na aparatu Ilab-600, dok su leukociti, eritrociti i hemoglobin rađeni metodom protočne citometrije na aparatu Coulter. Svim ispitanicima koji su prethodno bili na normalnoj ishrani bez alkohola i bez simptoma za hroničnu infekciju utvrđen je nivo rezistina. Krv je uzimana vakutanerom, punkcijom kubitalne vene, nakon čega se centrifugiranjem dobila plazma koja je skladištena na – 20 °C. Koncentracija rezistina određena je pomoću metode ELISA imunoesej, i opreme „ELISA human resistin“, proizvođača BioVendor Medicine, Czech Republic.

Kod svih ispitanika evidentirana je navika pušenja (samo aktivni pušači) i izmerena sistolna (SAP) i dijastolna (DAP) komponenta arterijskog krvnog pritiska. Od kliničkih karakteristika, na osnovu anamnestičkih i kliničko-laboratorijskih podataka, dobijeni su podaci o prisustvu DM i različitih formi KVO (ishemijska bolest srca, kongestivna srčana slabost i arterijska hipertenzija). Evidentirani su svi bolesnici sa najmanje III stepenom bubrežne slabosti (klirens kreatinina 30–59 mL/min). Klirens kreatinina izračunavan je prema formuli [9]:

$$\frac{(140 - \text{godine života}) \times \text{telesna masa (kg)}}{0{,}81 \times \text{koncentracija kreatinina } (\mu\text{mol/L})}$$

Podaci su analizirani primenom programa Instat (GraphPad Software Inc, San Diego, USA). Za testiranje razlike učestalosti korišćen je χ^2 test. Povezanost varijabli analizirana je primenom koeficijenta linearne korelacije. Vrednost $p < 0{,}05$ smatrana je statistički značajnom.

Rezultati

Studija je obuhvatila 102 bolesnika, 69 (67,6%) muškaraca i 33 (32,4%) žene, prosečne starosti 55 ± 9,3 godine. Pušača je bilo 36 (35,3%), sistolna komponenta arterijskog krvnog pritiska kod naših ispitanika imala je prosečnu vrednost 165 ± 17,1 mmHg, a dijastolna 100 ± 9,23 mmHg. Prosečna telesna masa ispitanika bila je 84 ± 14,3 kg, telesna visina 177 ± 7,4 cm, a ITM 28,5 ± 3,4 kg/m^2. Prosečna vrednost obima kukova bila je 100 ± 10,8 cm, obima struka 110 ± 17,4 cm, a njihov količnik 1,05 ± 0,14. Bolesti kardiovaskularnog sistema utvrđene su kod 37 (36,3%) ispitanika; DM je imalo 45 (44,1%), dok je BI verifikovana kod 20 (19,6%) učesnika u studiji (tabela 1).

Tabela 1

Bazični klinički i antropometrijski parametri ispitanika (n = 102)

Parametri	Vrednosti
Starost (godine), ($\bar{x}$ ± SD)	55 ± 9,3
Pol (m/ž), (n)	69/33
Pušač (da/ne), (n)	66/36
SAP (mmHg), ($\bar{x}$ ± SD)	165 ± 17,1
DAP (mmHg), ($\bar{x}$ ± SD)	100 ± 9,23
Telesna težina (kg), ($\bar{x}$ ± SD)	84 ± 14,3
Telesna visina (cm), ($\bar{x}$ ± SD)	177 ± 7,4
ITM kg/m^2, ($\bar{x}$ ± SD)	28,5 ± 3,4
Obim struka (cm), ($\bar{x}$ ± SD)	110 ± 17,4
Obim kukova (cm), ($\bar{x}$ ± SD)	100 ± 10,8
Odnos kuk/struk, ($\bar{x}$ ± SD)	1,05 ± 0,14
Komorbidne bolesti (n/%)	
kardiovaskularne bolesti	37/36,3
dijabetes melitus	45/44,1
bubrežna slabost	20/19,6

SAP – sistolni arterijski pritisak; DAP – dijastolni arterijski pritisak; ITM – indeks telesne mase

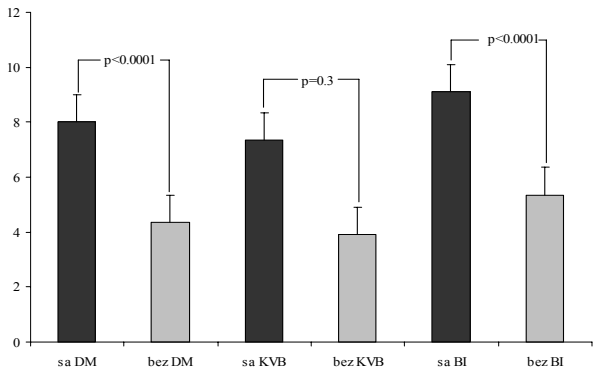
U tabeli 2 date su srednje vrednosti ispitivanih biohemijskih parametara. Koncentracije glukoze (8,7 ± 3,3 mmol/L),

uree (9,2 ± 3,05 mmol/L), kreatinina (113 ± 36,8 μmol/L), LDL holesterola (5 ± 0,8 mmol/L), triglicerida (2,8 ± 1,1 mmol/L) i rezistina (9,1 ± 6,01 μg/L) bile su više od normalnih vrednosti, dok je klirens kreatinina (70 ± 12,6 mL/min) bio ispod referentnih granica. Ostali ispitivani parametri imali su vrednosti u normalnom opsegu.

Tabela 2
Bazični laboratorijski parametari ispitanika (n = 102)

Parametri	Vrednosti ($\bar{x}$ ± SD)
Leukociti ($\times 10^9$/L)	7,3 ± 1,6
Eritrociti ($\times 10^{12}$/L)	3,8 ± 0,4
Hemoglobin (g/L)	115 ± 17,8
Glukoza (mmol/L)	8,7 ± 3,3
Urea (mmol/L)	9,2 ± 3,05
Kreatinin (μmol/L)	113 ± 36,8
Klirens kreatinina (mL/min)	70 ± 12,6
Holesterol (mmol/L)	7,8 ± 1,8
LDL holesterol (mmol/L)	5 ± 0,8
Triglyceridi (mmol/L)	2,8 ± 1,1
Ukupni proteini (g/L)	70 ± 5,05
Albumini (g/L)	42 ± 3,16
Rezistin (μg/L)	9,1 ± 6,01

Koncentracija rezistina kod bolesnika sa DM bila je 8 ± 4,5 μg/L, dok je grupa bolesnika bez ovog metaboličkog oboljenja imala srednje vrednosti rezistina od 7,5 ± 5,5 μg/L. Grupa bolesnika sa KVB imala je nivo rezistina u proseku 6,45 ± 4,99 μg/L, a bolesnici bez verifikovanih kardiovaskularnih oboljenja 8,2 ± 5,1 μg/L. Bolesnici sa BI imali su prosečan nivo rezistina 9,1 ± 6,01 μg/L, a grupa ispitanika bez BI 7,4 ± 4,2 μg/L. Statistički značajna razlika u odnosu na koncentraciju rezistina postignuta je između grupe bolesnika sa i bez DM ($p < 0,001$) i grupe bolesnika sa i bez BI ($p < 0,001$). Nije utvrđena statistički značajna razlika ($p = 0,3$) između grupe bolesnika sa i bez KVB (slika 1).



Sl. 1 – Koncentracija rezistina (μg/L) kod ispitanika sa i bez dijabetesa melitusa (DM), bubrežne insuficijencije (BI) i kardiovaskularnih bolesti (KVB)

U tabeli 3 prikazana je korelacija koncentracije rezistina u odnosu na broj manifestnih metaboličkih oboljenja po jednom ispitaniku. Bolesnici kod kojih je evidentirano jedno metaboličko oboljenje imali su u proseku koncentraciju rezistina od 6,07 ± 1,93 μg/L, bolesnici sa dva metabolička oboljenja 7,39 ± 2,19 μg/L, bolesnici koji su imali sva tri metabolička oboljenja imali su prosečne vrednosti rezistina 11,43 ± 6,19 μg/L. U odnosu na nivo rezistina prema broju metaboličkih oboljenja po jednom bolesniku postignuta je statistički značajna razlika (F = 11,80; DF = 4,61; $p < 0,001$).

Tabela 3
Koncentracija rezistina i broj bolesti prisutnih kod jednog ispitanika

Broj metaboličkih oboljenja po jednom bolesniku	Rezistin (μg/L) $\bar{x}$ ± SD
1	6,07 ± 1,93
2	7,39 ± 2,19
3	11,43 ± 6,19

Diskusija

Povećana koncentracija rezistina u nekim istraživanjima okrivljuje se za pojavu KVO, ali se njihova prediktivna vrednost tek mora potvrditi [8]. Naša nastojanja u ovom radu bila su da utvrdimo značaj koncentracije rezistina kao biomarkera kod epidemiološki važnih metaboličkih bolesti. Rezultati našeg istraživanja pokazali su da bolesnici sa KVO nemaju povišene vrednosti rezistina u odnosu na grupu bolesnika bez kardiovaskularnog morbiditeta. Ostaje, međutim, pitanje eventualne detekcije postojanja različitih izomera rezistina, što upućuje na to da su, u ovom slučaju, neophodni i neki dodatni markeri kao precizniji prediktori KVB [11].

Incidencija i prevalencija hronične BI u Sjedinjenim Američkim Državama poprima alarmantnu stopu. Istraživanja pokazuju da postoji veći rizik od aterosklerotskih komplikacija i mortaliteta kod bolesnika sa blagom do umerenom formom hronične BI, u odnosu na osobe bez izražene BI [12, 13]. Tačna fiziološka uloga rezistina nedovoljno je proučena, a podaci o uticaju na bubrežne bolesti veoma su oskudni. Diez i sar. [12] našli su značajno više vrednosti rezistina kod bolesnika lečenih hemodijalizama i peritoneumskim dijalizama, u odnosu na one koji svoju BI regulišu ne tako radikalnim terapijskim protokolom, verovatno zbog toga što je difuzijom nemoguća eliminacija molekula veličine 12,5 kDa. Ipak, ostaje podatak koji se potvrđuje i u mnogim studijama da, što je funkcija bubrega niža, to je koncentracija rezistina veća. Epidemiološke studije koje istražuju uticaj gojaznosti na ishod bolesnika sa hroničnom BI su u kontradiktornosti sa nekoliko dobro dizajniranih studija koje čak sugerišu da gojaznost kod bolesnika na hemodijalizi predstavlja prediktivni i zaštitni faktor preživljavanja ovih bolesnika [13, 14]. Rezultati našeg istraživanja pokazali su da je rezistin, kao adipozospecifičan peptid, statistički značajno viših koncentracija kod bolesnika sa izraženom bubrežnom slabošću. To navodi na zaključak da postoje i neki drugi mehanizmi koji bi mogli biti uključeni u metabolizam ovog hormona kod bolesnika čiji stepen BI ne zahteva neki od vidova dijalizne depuracije. Zbog toga, neophodne su dobro dizajnirane studije koje bi dodatno razjasnile mehanizam eliminacije rezistina i utvrdile njenu prediktivnu vrednost na eventualnu progresiju bubrežne slabosti.

Podaci o uticaju adipozospecifičnih hormona na insulin prilično su kontroverzni. Neki autori navode udruženost povećanog nivoa rezistina i prekomerne telesne mase, i to njenog visceralnog odeljka, kao i kalcifikacije koronarnih krvnih sudova [15, 16]. Istraživanja na humanoj populaciji o ulozi rezistina trebalo bi da donesu određene koristi u smislu novih terapijskih procedura kod DM i gojaznosti [17]. Naša studija je kod bolesnika sa DM utvrdila statistički značajno veću koncentraciju rezistina, u odnosu na ispitanike koji nisu imali ovo metaboličko oboljenje, bez obzira na njihove antropometrijske karakteristike, što, takođe, može da sugeriše postojanje različitih izoformnih oblika rezistina.

Rezultati našeg istraživanja pokazuju da se koncentracija rezistina povećava sa povećanjem broja metaboličkih oboljenja po jednom bolesniku, što navodi na razmišljanje da veći broj metaboličkih bolesti uslovljava više vrednosti ovog peptida. Naime, utvrdili smo statistički značajnu povezanost broja bolesti koje su prisutne kod jednog ispitanika i koncentracije rezistina.

Zaključak

Antropometrijske vrednosti naših ispitanika ukazuju na značajnu intraabdominalnu distribuciju masnog tkiva i prekomernu uhranjenost. Visoka koncentracija rezistina značajno karakteriše bolesnike koji imaju i BI i DM. Ipak, naša studija utvrdila je i određene dileme, pa su neophodna dodatna istraživanja kako bi se do kraja razjasnila uloga rezistina na pojavu i razvoj metaboličkih oboljenja koja imaju veliki uticaj na zdravlje ljudi.

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Perimenstrual headache: migraine without aura or premenstrual syndrome symptom?

Perimenstrualna glavobolja: migrena bez aure ili simptom predmenstrualnog sindroma?

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Abstract

Background/Aim. Definition of menstrual migraine as a specific clinical entity or, maybe, migraine headache with menstrually related occurring, still remains unresolved question. The aim of this study was to investigate if perimenstrual headache in our patients fulfills diagnostic the International Classification of Headache Disorders (ICHD) criteria for migraine without aura or represents a different type of headache which is the symptom of premenstrual syndrome (PMS). **Methods.** The study included 50 women with headache in perimenstrual period in at least two out of three menstrual cycles, during the last year or longer. Two questionnaires, a questionnaire for headache and a questionnaire for PMS, were used. **Results.** The majority of all the examined women, 29 of them, had migraine and PMS and 9 women had migraine without PMS. Headache in 38 (76.0%) patients fulfilled diagnostic criteria for menstrual migraine, (26 and 12 women had pure menstrual migraine and menstrually related migraine respectively). Intensity of PMS was not different in a groups of women with different types of headache ($p = 0.184$): a total number of PMS symptoms was 8.2 ± 4.6 in the group with pure menstrual migraine, 10.8 ± 3.9 in the group with menstrually related migraine and 10.8 ± 6.3 in the group with non-migraine headache. **Conclusion.** This study shows that headache, occuring in perimenstrual period, is not always migraine, but could fulfill criteria for tension-type headache, as well. Specific characteristics of perimenstrual headache, which could distinguish it as a symptom of PMS, were not found. Expected relation in time of headache onset and menarche was not confirmed.

Key words:
migraine disorders; headache; diagnosis; questionnaires; premenstrual syndrome; quality of life; psychology, medical.

Apstrakt

Uvod/Cilj. Još uvek nije jasno da li menstrualna migrena predstavlja zaseban klinički entitet ili je reč o migrenskoj glavobolji koja se specifično javlja u menstrualnom periodu. Cilj ovog istraživanja bio je da se utvrdi da li perimenstrualna glavobolja ispitanih bolesnica ispunjava dijagnostičke kriterijume Međunarodne klasifikacije glavobolja (MKG) za migrenu bez aure ili predstavlja glavobolju drugih, nemigrenskih osobina koja se javlja kao simptom predmenstrualnog sindroma (PMS). **Metode.** Istraživanjem je bilo obuhvaćeno 50 žena sa glavoboljom u perimenstrualnom periodu u najmanje dva od tri menstrualna ciklusa tokom poslednjih godinu dana ili duže. Istraživanje je urađeno pomoću dva strukturisana upitnika: Upitnika za glavobolju i Upitnika za PMS. **Rezultati.** Najveći broj ispitivanih žena, njih 29, imalo je migrenu i PMS, dok je migrena bez PMS-a zabeležena kod devet ispitanica. U ispitivanoj grupi, glavobolja kod 38 žena (76,0%) ispunjavala je dijagnostičke kriterijume za menstrualnu migrenu, od čega je 26 žena imalo čistu menstrualnu migrenu, a 12 migrenu povezanu sa menstruacijom. Stepen izraženosti PMS-a nije se razlikovao među grupama žena sa različitim tipovima glavobolje ($p = 0,184$): žene sa čistom menstrualnom migrenom imale su prosečno 8,2 ± 4,6, žene sa menstrualno povezanom migrenom 10,8 ± 3,9, a žene sa glavoboljom nemigrenskih osobina 10,8 ± 6,3 simptoma PMS-a. **Zaključak.** Ovim istraživanjem pokazano je da glavobolja koja se javlja u perimenstrualnom periodu nije obavezno migrena, već se javlja i kao glavobolja tenzionog tipa. Posebne osobine perimenstrualne glavobolje, na osnovu kojih bi ona mogla biti tumačena kao simptom PMS-a, nisu uočene. Ovim istraživanjem nije potvrđena očekivana vremenska povezanost menarhe i pojave prve glavobolje.

Ključne reči:
migrena; glavobolja; dijagnoza; upitnici; premenstrualni sindrom; kvalitet života; psihologija, medicinska.

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Introduction

Migraine is a type of headache with recurrent attacks of unilateral, pulsating pain of high intensity which aggravate with physical activity and is accompanied by photo- and/or phonophobia and nausea and/or vomiting. Menstruation is claimed to be one of the trigger factors for migraine attack in more than half of in-hospital examined patients with migraine and, according to population based studies, in more than a quarter of women with migraine [1]. Headache gets worsen in 60% of women with migraine during the perimenstrual period, e.g. two days before and the first three days of menstrual bleeding, and some women, in smaller percent, report the headache attacks occurring exclusively in this period [2, 3]. Menstrual migraine exists as a pure menstrual and menstrually related migraine [4]. Patients with pure menstrual migraine report migraine attacks exclusively two days before or during the first three days of menstrual bleeding, and women with menstrually related migraine have additional pain attacks in other time of menstrual cycle, in at least two out of three menstrual cycles, for both diagnosis.

Premenstrual syndrome (PMS), according to the American College for Gynecology and Obstetristics, consists of phsysical and psychological symptoms and signs, occurring during at least five days before menstrual bleeding and completely resolving in four days after the bleeding onset, in at least three consecutive menstrual cycles [5]. More than 50% of women with PMS has headache as one of the symptoms [6], but, exact clinical characterisation of type of this headache was not given.

There is no enough data which explain if menstrual migraine is specific type of headache, or simply migraine without aura, occurring in menstrual period. Also, there is none specific biomarker which can be used for differentiation menstrual and other types of migraine.

The aim of this study was to investigate if perimenstrual headache in our patients corresponds with diagnostic the International Classification of Headache Disrders (ICHD) criteria for migraine without aura or represents a different type of headache which is the symptom of PMS.

Methods

This study included 50 women with headache in perimenstrual period in at least two out of three menstrual cycles, during the last year or longer. All the patients were recruited in the Headache Center, Institute of Neurology, Belgrade, in the period from January 2006 to January 2008. Exclusion criteria for this study were pregnancy, ongoing hormonal or prophylactic therapy of migraine. Cases of symptomatic headache were excluded by neurological examination, laboratory blood testing, ophthalmological examination, electroencephalography (EEG), computed tomography (CT) and nuclear magnetic resonance (NMR) brain examinations. Two questionnaires, a questionnaire for headache and a questionnaire for PMS, were used.

Clinical characteristics of perimenstrual headache were estimated by, for this survey designed, the original Questionnaire for Headache (Attachment 1), and diagnosis of migraine without aura was given according to IHC diagnostic criteria. Frequency of headache attacks was recorded in perimenstrual period, but in other time of menstrual cycle as well and quantified as number of days with headache in one month period. Intensity of pain was estimated using the verbal analog scale (VAS, with 0 for notification of total absence of pain, and 10 for the pain with maximum of its intensity) (Attachment 2). According to data recorded using the Questionnaire for Headache, all patients were divided into three groups: women with pure menstrual migraine, with menstrually related migraine and with non-migraine headache. In these groups, we analysed the intensity of PMS and demographic features of patients.

Diagnosis of PMS was given using the Questionnaire for PMS, modification of the Moos's Menstrual Distress Questionnaire [7]. Using this questionnaire, we recorded phsysical and psychological symptoms and signs, occurring during at least five days before menstrual bleeding, persisting or vanishing during menstruation and completely resolving in four days after the bleeding onset, in at least three consecutive menstrual cycles. Intensity of PMS was quantified as a sum of points given for every symptom and sign. A maximum of points was 28. Diagnosis of severe PMS was given to patients with more than 20 points, of moderate PMS to those with 11 to 20 points, of mild PMS to those with 6 to 10 points, and a sum of less than 6 points indicated that a patient did not have PMS. According to the results of this questionnaire, all the examined women were divided into two groups: women with and without PMS. In these groups we analysed clinical characteristics of headache and demographic features of patients.

Statistical analyses of data was done in SPSS v.16.0.pm programme, using Student's *t*-test, ANOVA and χ^2 test. Test value $p < 0.05$ was considered to be statistically significant.

Results

According to diagnosis of migraine without aura nad PMS, four groups have been formed: women with migraine and PMS, with migraine and without PMS, and with non-migraine headache and PMS, and with non-migraine headache and without PMS. The majority of examined women, 29 of them, had migraine and PMS, and 9 patients had migraine without PMS. Non-migraine headache with PMS was present in 10 women, and non-migraine headache without PMS was recorded in only two cases. Demographic features of patients and the clinical characteristics of headache in these groups are given in Table 1.

Demographic features of patients and characteristics of headache were not significantly different in the groups of patients with perimenstrual headache (Table 1).

In 38 (76.0%) of all the examined women, headache fulfilled the IHC diagnostic criteria for menstrual migraine, with diagnosis of pure menstrual migraine in 26, and menstrually related migraine in 12 of them. Demographic features of patients and the clinical characteristics of headache in women with menstrual migraine are given in Table 2.

Table 1

Demographic features and clinical characteristics of headache in women with perimenstrual headache according to diagnosis of migraine and PMS

Parameters	Groups of women examined					
	all women	M and PMS	M without PMS	NM and PMS	NM without PMS	*p*
n (%)	50 (100)	29 (58)	9 (18)	10 (20)	2 (4)	
Age at the time of examination ($\bar{x} \pm SD$)	36.5 ± 8.0	35.9 ± 7.5	39.9 ± 9.4	35.5 ± 8.9	34.5 ± 6.4	0.574
Age at the time of headache onset (years), ($\bar{x} \pm SD$)	20.7 ± 9.1	21.3 ± 8.7	19.9 ± 10.4	18.2 ± 9.3	27.5 ± 9.2	0.567
Age at the time of menarche (years), ($\bar{x} \pm SD$)	12.6 ± 1.2	12.6 ± 1.2	12.6 ± 1.1	12.8 ± 0.9	11.0 ± 1.4	0.275
Frequency (days in one month), ($\bar{x} \pm SD$)	4.5 ± 3.8	4.1 ± 3.1	3.8 ± 4.3	6.8 ± 4.7	3.0 ± 1.4	0.189
Duration of attack (h), ($\bar{x} \pm SD$)	35.7 ± 27.5	39.9 ± 27.4	37.9 ± 32.9	25.8 ± 22.9	14.0 ± 14.1	0.360
Education – college and more; n (%)	28 (56.0)	15 (51.7)	5 (55.6)	8 (80.0)	0	0.165
Nulliparous; n (%)	16 (32.0)	11 (37.9)	2 (22.2)	3 (30,0)	0	0.610

M – migraine; NM – non-migraine headache; PMS – premenstrual syndrome; $p < 0.05$ – statistically significant

Table 2

Demographic features and clinical characteristics of headache in women with menstrual migraine

Parameters	Groups of women		
	pure menstrual migraine	menstrually related migraine	*p*
n (%)	26 (68.4)	12 (31.6)	
Age at the time of examination (years), ($\bar{x} \pm SD$)	38.58 ± 8.02	33.00 ± 7.03	0.046
Age at the time of headache onset (years), ($\bar{x} \pm SD$)	22.96 ± 9.17	16.67 ± 7.28	0.044
Age at the time of menarche (years), ($\bar{x} \pm SD$)	12.50 ± 1.175	12.75 ± 1.29	0.558
Frequency (days in month), ($\bar{x} \pm SD$)	3.35 ± 2.98	5.42 ± 3.85	0.078
Duration of attack (h), ($\bar{x} \pm SD$)	39.54 ± 28.99	39.17 ± 28.20	0.971
Number of PMS symptoms (0–28), ($\bar{x} \pm SD$)	8.23 ± 4.57	10.83 ± 3.90	0.097
Education – college and more n (%)	13 (50.0)	7 (58.3)	0.632
Nulliparous, n (%)	6 (23.1)	7 (58.3)	0.033

PMS – premenstrual syndrome; $p < 0.05$ – statistically significant

Compared with women with menstrually related migraine, women with pure menstrual migraine were older at the time of examination and at the time of headache onset, as well. Age at the time of menarche was similar in all the examined women. Age at the time of headache onset and menarche in women with menstrual migraine is given in Figure 1.

Characteristics of headache (duration of attack and frequency), intensity of PMS and education level were not significantly different in the examined groups, but the percent of women not having children at the time of examination was higher in the group with menstrually related migraine (Table 2).

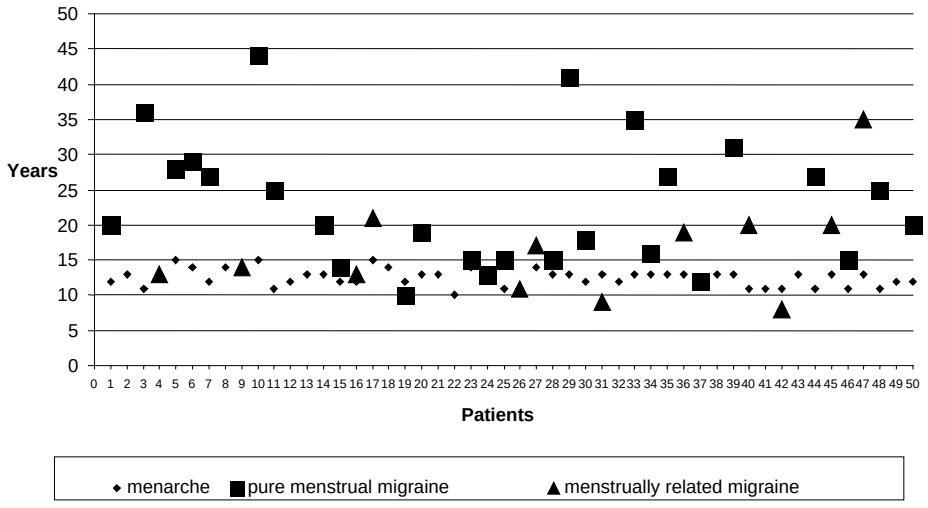

Fig. 1 – Age at the time of headache onset and menarche in women with pure menstrual and menstrually related migraine

Intensity of PMS was not different in the groups of women with different types of headache ($p = 0.184$): total number of PMS symptoms was 8.2 ± 4.6 in the group with pure menstrual migraine, 10.8 ± 3.9 in the group with menstrually related migraine and 10.8 ± 6.3 in the group with non-migraine headache.

Discussion

Since ICHD [4] does not recognize menstrual migraine as separate entity, the Appendix to ICHD, as a suggestion for the next revision of classification, gives diagnostic criteria for two types of menstrual migraine, pure menstrual and menstrually-related migraine. Diagnoses of specific types of headache in this study were put according to these diagnostic criteria. In the patients with perimenstrual headache which fulfills diagnostic criteria for migraine without aura, more than 60% had pure menstrual, and about one third of them had menstrually related migraine. Results of several studies [8–13] suggest that in population of women with migraine without aura, menstrually related migraine has higher frequency of 35% to 54%, and pure menstrual migraine is reported in 4% to 21% cases. Predomination of pure menstrual migraine in our results can be explained by a specific selection of patients in the Headache Center, as a third level health care center. Secondly, using inclusion criteria of having headache in perimenstrual period, we got the results of predomination of pure menstrual migraine in patients with migraine in perimenstrual period, while previous investigation's subject was general population of women with migraine without aura.

The majority of women, two third of them, had migraine without aura, and one third had non-migraine headache. There is no sufficient data indicating that headache occuring in perimenstrual period has to be migraine without aura only. In the last edition of ICHD, only one type of primary headaches is related to perimenstrual period, migraine without aura with its two forms: pure menstrual and menstrually related migraine. Time relation of perimenstrual period and headache attacks in patients with tension type headache and cluster headache has been reported as a result of only few studies [14, 15]. In this study, women with headache attacks not fulfilling ICHD diagnostic criteria for migraine without aura were classified in the group with non-migraine headache. In that way, non-migraine headache attacks fulfilled ICHD diagnostic criteria for tension type headache, given in the ICHD as a negation of migraine criteria, and the conclusion is that one third of women in this study had, in fact, tension type headache in perimenstrual period. In the study which examined 45 women with headache and PMS, 60% of them had migraine without aura, and about 30% had tension type headache [16]. This data are in accordance with suggestions of some authors that menstrual tension type headache exists as a separate entity and because of that deserves its place and definition in ICHD [14].

Most of women with perimenstrual headache had mild PMS. Different types of headache were not related to intensity of PMS, meaning that PMS was not more severe in women with non-migraine headache comparing to those with migraine. Only few studies investigated if some specific type of headache in perimenstrual period is dominant in patients with PMS, so, the contribution of our study is the data that migraine without aura, as the most frequent type of headache in perimenstrual period, is not dependent on intensity of PMS.

Women with pure menstrual migraine, comparing to women with menstrually related migraine, were older at the time of first examination. This data suggests that women with headache occurring not only in the perimenstrual period, but additionally in other time of menstrual cycle, are more disabled by a high frequency of headache attacks, have lower quality of life and contact doctor for help earlier. Also, women with pure menstrual migraine were older at the time of headache onset, while age of menarche was similar in all women. According to the present knowledge, authors of this paper are not able to give explanation for this result. Time gap between age at the time of menarche and age at the time of headache onset in women with menstrual migraine, is not reported by other authors. Hershey [17] reports time relation between first attack of menstrual migraine and menarche, but Kröner-Herwig and Vath [18] do not find a connection between menarche and increased frequency of headache in girls in puberty. There is a lack of explanation for even more significant time gap between menarche and headache onset in women with pure menstrual migraine, comparing to those with menstrually related migraine.

A significantly higher percent of women with pure menstrual migraine already had children at the time of examination, what can be connected to age at the time of first examination.

Conclusion

This study shows that headache occurring in perimenstrual period is not always migraine, but could fulfill diagnostic criteria for tension-type headache, as well. Specific characteristics of perimenstrual headache, which could distinguish it as a symptom of PMS, were not found. An expected relation in time of headache onset and menarche was not confirmed in this study.

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Attachment 1

Questionnaire for Headache

Name _____ **Number** _____

Date of birth: _____
Phone:_____
Education level: primary school / high school / university degree
Profession: _____

Children yes / no, how many children _____
In what way did pregnancy affect migraine _____
Migraine after birth during breastfeeding and after breastfeeding _____

Height _____cm Weight _____kg
Right handed / left handed / Ambidexter

Age of headache onset: _____
Age of menstruation onset: _____

Inclusive criteria:
Headache in perimenstrual period in 2 out of 3 menstrual cycles yes / no

Exclusive criteria:

Irregular menstrual cycle yes / no
Hormonal therapy yes / no
Contraceptives yes/no
Prophylactic therapy of migraine yes / no
Pregnancy yes / no
Breastfeeding yes / no
Other acute or chronic disease yes / no

Frequency: _____ days in month

Connection with perimenstrual period:

Headache occurs _____ days before menstruation (from day 1)
During ovulation yes / no (On what day after day 1 _____
During menstruation yes / no (On what day after day 1 _____
After menstruation yes / no (On what day after day 1 _____

Intensity of pain: _____ on the scale from 0 to 10 (untreated)

WORSENING WITH PHYSICAL ACTIVITY YES / NO

QUALITY OF PAIN: sharp dull pulsating (constant)

LOCALISATION: Unilateral – changing sides,
Unilateral – without changing sides, Left/right
Bilateral
Diffuse

DURATION OF HEADACHE: _____ hours

ACCOMPANING SYMPTOMS:
photo ___%, phono____% , osmophobia ____%, nausea ____%, vomiting _____% (for each - percent of migraine attacks)

PRODROMS:
hunger/ yawing / tiredness / swelling / lack of mental concetration / mood changes /
other:_____

PROVOCATIVE FACTORS:
Stress / hunger / lack of sleep / too much of sleep / tiredness / type of food / medications /
other:_____

THERAPY:
Acute:
Which medication you usually use for stopping the attack? _____

Prophylactic if tried beforehand:_____
Do you use any kind of medication daily? _____

Other medication (not for headache): _____

HABITS: smoking yes / no , alcohol yes / no, coffee yes / no

FAMILY ANAMNESIS: Does someone from your family suffer from headache yes / no (exact relation to the subject) _____
Are those headaches similar to yours? yes / no

Date: _ _ / _ _/ _ _ _ _

Attachment 2

Questionnaire for PMS

0 - no symptoms
1 - mild symptom
2 - moderate symptom
3 - severe symptom

SYMPTOM	BEFORE	DURING menstrual bleeding	AFTER
HEADACHE			
LUMBAL PAIN			
TIREDNESS			
NUMBNESS OF THE BODY			
INSOMNIA			
FORGETFULNESS			
CONCENTRATION DIFFICULTIES			
ABSTRACTION			
CLUMSINESS			
WORKING DIFFICULTIES			
FAINTING			
SWEATING			
FREQUENT URINATION			
REDUCE INTEREST IN SEX			
HUNGER FOR SWEETS			
NAUSEA, VOMITING			
WEIGHT GAIN			
BREAST PAIN			
EYELID EDEMA			
CRYING			
DEPRESSION			
MOOD CHANGES			
ENHANCEMENT OF APPETITE			
VISUAL DISTURBANCES			
PALPOTATIONS			
ACNE			
FREQUENT INFECTIONS			
ALERGIC REACTIONS			

NAME _____

NUMBER _____

DATE _____

ORIGINALNI ČLANAK UDC 618.14–006–008.9–092.18–097

Imunocitohemijske karakteristike submukoznih mioma materice

Immunocytochemical characteristics of submucosal uterine myomas

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Apstrakt

Uvod/Cilj. Miomi kao najzastupljeniji benigni tumori materice već decenijama istražuju se sa aspekta različitih bazičnih i kliničkih disciplina. Uprkos tome, njihova patogeneza još uvek nije sasvim razjašnjena. Cilj ove studije bio je utvrđivanje imunocitohemijskih karakteristika glatkih mišićnih ćelija i komponenti vezivnog tkiva u sastavu submukoznih mioma uterusa. **Metode.** Analizirano je 25 uzoraka submukoznih mioma materice dobijenih intraoperativno, nakon abdominalne histerektomije prema Aldridge-u. Uzorci su fiksirani u 4% formalinu i kalupljeni u paraplastu. Rezovi debljine 5 μm bojeni su imunohistohemijskom tehnikom DAKO LSAB+/HRP koja je primenjena za identifikaciju sledećih antigena: α-glatkomišićni aktin–α-SMA, vimentin, dezmin, CD34, CD45 i PCNA (DAKO specifikacija). **Rezultati.** Pokazano je da se u sastavu submukoznih mioma materice nalaze glatke mišićne ćelije imunoreaktivne na α-SMA i dezmin, ali i jedan broj glatkih mišićnih ćelija imunoreaktivnih na α-SMA i vimentin. Neke od vimentin-imunoreaktivnih ćelija pokazuju i imunoreaktivnost na PCNA. U sastavu vezivne strome zastupljeni su CD34 imunoreaktivni fibroblasti i krvni sudovi različitog prečnika. Ispitivanjem distribucije CD45 antigena, na uzorcima je zapažena umerena ili slaba reakcija. **Zaključak.** U sastavu submukoznih mioma uterusa nalaze se glatke mišićne ćelije visokodiferentovanog kontraktilnog fenotipa (imunoreaktivnost na α-SMA i dezmin) kao i glatke mišićne ćelije sintetskog fenotipa koje proliferišu (imunoreaktivnost na α-SMA i vimentin; imunoreaktivnost na PCNA). U submukoznim miomima uterusa prisutna je velika količina vezivnog tkiva nastalog kao produkt sinteze fibroblasta koji se prema svojim imunohistohemijskim karakteristikama jasno razlikuju od glatkih mišićnih ćelija sintetskog fenotipa.

Ključne reči:
materica, neoplazme; miom; miociti, glatki mišić; vimentin; desmin.

Abstract

Background/Aim. Myomas of the uterus, the most common benign tumors, have been studied for decades from the aspects of different basic and clinical disciplines. Despite this fact, their pathogenesis is still poorly understood. The aim of this study was to determine immunocytochemical characteristics of smooth muscle cells and connective tissue components of submucosal myomas of the uterus. **Method.** During the course of this study, 25 samples of submucosal myomas of the uterus were analyzed, all of them obtained during the surgery, after abdominal histerctomy by Aldridge. The samples were fixed in 4% formalin and embedded in paraffin. Sections of 5 μm thickness were stained immunocytochemically using the DAKO LSAB+/HRP technique to identify α-smooth muscle actin (α-SMA), vimentin, desmin, CD34, CD45, CD68 and PCNA (DAKO specification). **Results.** Our results suggest that submucosal myomas of the uterus are build-up of smooth muscle cells which are immunoreactive to α-SMA and desmin, but also to a certain number of smooth muscle cells which are immunoreactive to α-SMA and vimentin. Some of vimentin-immunoreactive cells also show an immunoreactivity of PCNA. In the build-up of connective stroma CD34-immunoreactive fibroblasts and neovascular formations are also present. By examining the distribution of CD45 antigen, at all the analyzed samples we observed a weak reaction. **Conclusion.** Submucosal myomas of the uterus are made-up of smooth muscle cells of the highly differentiated contractile phenotype (α-SMA- and desmin-immunoreactivity), as well as smooth muscle cell of the synthetic phenotype which proliferate (α-SMA-, vimentin- and PCNA-immunoreactivity). In submucosal myoma of the uterus there is a significant presence of connective tissue as a result of synthetic activity of fibroblasts, which clearly differ in their immunocytochemical characteristics from smooth muscle cells of the synthetic phenotype.

Key words:
uterine neoplasms; myoma; myocytes, smooth muscle; vimentin; desmin.

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Uvod

Miomi materice, jasno ograničeni benigni tumori glatkomišićnog tkiva, najčešće se javljaju u periodu između 35. i 50. godine života, a prisutni su kod 35–70% žena [1]. Nakon menopauze, učestalost ovih tumora opada i iznosi svega 4%. Podjednako su zastupljeni kod žena koje su rađale i kod onih koje nisu. Uzroci nastanka mioma su mnogostruki i još uvek nedovoljno potvrđeni i dokazani. Smatra se da nasleđe, rasna pripadnost i tip konstitucije mogu da budu predisponirajući faktori razvoja mioma [2]. U novije vreme smatra se da je hormonska stimulacija jedan od najznačajnijih činilaca u patogenezi ovog oboljenja [3]. U saglasnosti sa ovom hipotezom su i rezultati brojnih savremenih kliničkih istraživanja kojima je dokazano da miomi materice ne nastaju pre puberteta, da se povećavaju tokom trudnoće i da se smanjuju i regrediraju tokom menopauze [4]. Međutim, prema nekim istraživanjima, miomi ne nastaju direktnom hormonskom stimulacijom, već posrednim dejstvom hormona na stepen prokrvljenosti uterusa, što je potvrđeno i činjenicom da, ukoliko tumori preuzimaju vaskularizaciju iz nekog susednog organa, ne dolazi do njihove regresije u uslovima smanjene hormonske stimulacije [5].

Prema lokalizaciji, miomi materice dele se na miome tela (oko 92%) i miome istmusa materice (oko 8%), dok su miomi cerviksa veoma retki (svega 0,25–0,35%). Prema pravcu rasta, uobičajeno se dele na submukozne, intramuralne, supserozne i intraligamentarne [1, 6]. Grupi submukoznih mioma (*leiomyoma submucosum*) pripadaju tumori koji se svojim rastom pomeraju prema materičnoj duplji i smeštaju pod sluznicu. Svojim položajem pritiskaju i istežu endometrijum izazivajući njegovu atrofiju i nekrozu. Kontrakcijama mišićnog sloja submukozni miomi mogu da dobiju polipoidnu formu. Ukoliko dođe do kompromitovanja cirkulacije u peteljci i nekroze, tumor može da bude izbačen kroz cervikalni kanal u vaginu (*leiomyoma uteri ad vaginum natum-myoma nascens*) [2]. Submukozni miomi javljaju se kod 5–10% ukupnog broja mioma [7]. Uzrokuju različite kliničke simptome, od kojih je najznačajniji metroragija sa posledičnom anemijom. Histerektomija predstavlja uobičajen hirurški tretman u lečenju submukoznih mioma uterusa, ali se u novije vreme primenjuje i manje invazivni tretman histeroskopske miomektomije [7–9].

Uprkos tome što se već decenijama istražuju sa aspekta različitih bazičnih i kliničnih disciplina, patogeneza mioma materice još uvek nije sasvim razjašnjena. Dugo se smatralo da miomi vode poreklo od glatkih mišićnih ćelija materice ili od fibroblasta [10]. Međutim, postoje i pretpostavke da miomi mogu da nastanu i od glatkih mišićnih ćelija koje se nalaze u zidovima krvnih sudova materice [5, 10]. Kako u savremenoj literaturi postoje različite hipoteze o nastanku i razvoju mioma precizno utvrđivanje imunocistohemijskih i morfofunkcionalnih karakteristika heterogenih ćelijskih populacija u njihovom sastavu doprinelo bi boljem razumevanju patohistoloških mehanizama koji dovode do nastanka mioma materice.

Cilj ovog istraživanja bio je utvrđivanje histološke organizacije submukoznih mioma materice, pre svega utvrđivanje fenotipskog statusa glatkih mišićnih ćelija mioma, utvrđivanje njihove proliferativne aktivnosti, kao i utvrđivanje sastava ekstracelularnog matriksa strome mioma, stepena neovaskularizacije, kao i leukocitne infiltracije.

Metode

Za potrebe ovog istraživanja korišćeno je 25 uzoraka submukoznih mioma uterusa, dobijenih intraoperativno, nakon abdominalne histerektomije prema Aldridge-u. Uzorci su prikupljani u Bolnici za ginekologiju i akušerstvo Kliničko-bolničkog centra „Zvezdara“ u Beogradu, od bolesnica koje su imale nepravilna krvarenja, obilna ili produžena ili i obilna i produžena krvarenja. Istraživanje je obavljeno po dobijanju informisanog pristanka bolesnika u skladu sa Helsinškom deklaracijom i preporukama Svetske zdravstvene organizacije (SZO) za eksperimente na humanom materijalu, nakon dobijanja saglasnosti Etičkog komiteta.

Tkivo je fiksirano u 4% neutralnom puferovanom formalinu 24 časa i kalupljeno u paraplastu. Rezovi debljine 5 μm montirani su na posebne visokoadherentne pločice *SuperFrost* i sušeni na temperaturi od 56 ºC u toku 1 sata. Procedura imunohistohemijskog bojenja podrazumevala je postupke demaskiranja antigena, blokiranja endogene peroksidaze, inkubiranja preparata sa primarnim antiserumom i postupak izvođenja imunohistohemijske metode – LSAB+/HRP.

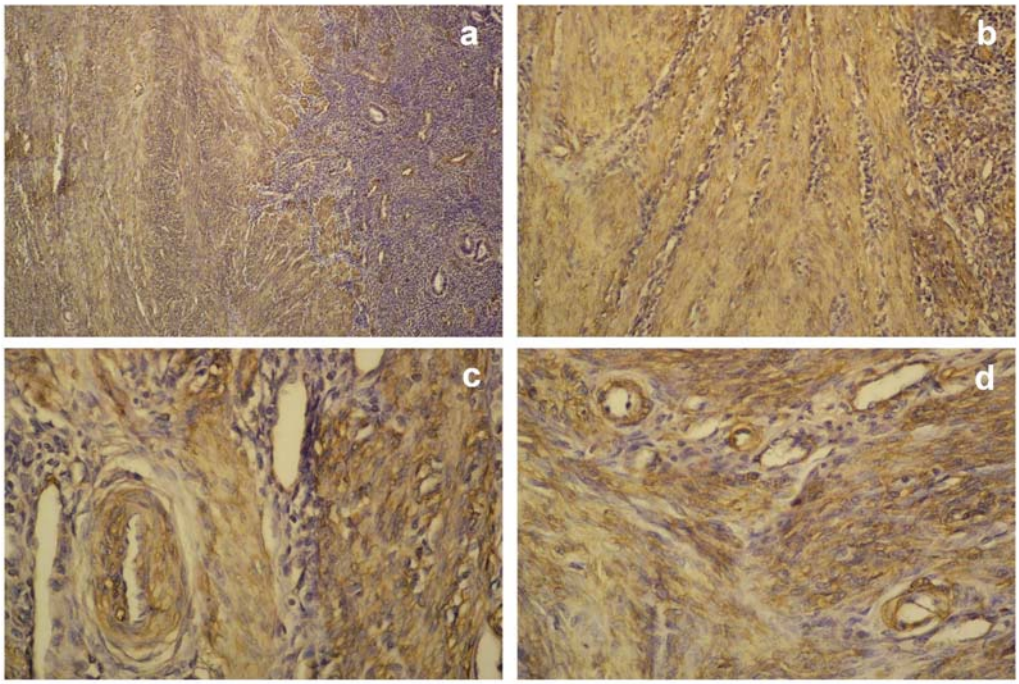
Nakon deparafinizacije i rehidratacije tkivnih preseka vršeno je demaskiranje antigena. Za ovaj proces korišćena je kućna mikrotalasna pećnica Molinex Compact. Demaskiranje antigena vršeno je u 0,1 M citratnom puferu, pH 6,0, na 800 W, u trajanju od 21 minut. Nakon demaskiranja antigena usledilo je blokiranje endogene peroksidaze 3% vodenim rastvorom H_2O_2 u trajanju od 10 minuta, a nakon toga nanošenje primarnog antitela i inkubacija u trajanju od 1h na sobnoj temperaturi, u vlažnoj komori, na način koji je prethodno opisan [11, 12]. Korišćena su sledeća primarna antitela u datim razblaženjima (prema DAKO specifikaciji): vimentin (1:100), α-glatkomišićni aktin (1:25), dezmin (1:10), CD34 (1:25), CD45 (zajednički antigen leukocita (*Leucocyte Common Antigen* – LCA) (1:50) i PCNA (1:25). Nakon inkubacije sa primarnim antitelom, usledila je inkubacija, prvo sa biotiniziranim vezujućim antitelom, a zatim sa streptavidinom obeleženim peroksidazom. Postupak je završen inkubacijom preseka u mešavini supstrat-hromogena (*DAKO liquid DAB + Substrate – Chromogen system K0679*) 5 min na sobnoj temperaturi. Kao opšti rastvarač antiseruma i sredstvo za ispiranje između različitih koraka u toku imunohistohemijske procedure bojenja korišćen je 0,1M fosfatni pufer pH 7,4. Ćelijska jedra bojena su Mayer-ovim hematoksilinom.

Sva imunohistohemijska bojenja izvedena su uz kontrolu kvaliteta i specifičnosti bojenja, primenom pozitivnih i negativnih kontrola prema propozicijama UK NEQAS (UK *National External Quality Assessment for Immunocytochemistry* – UK NEQAS).

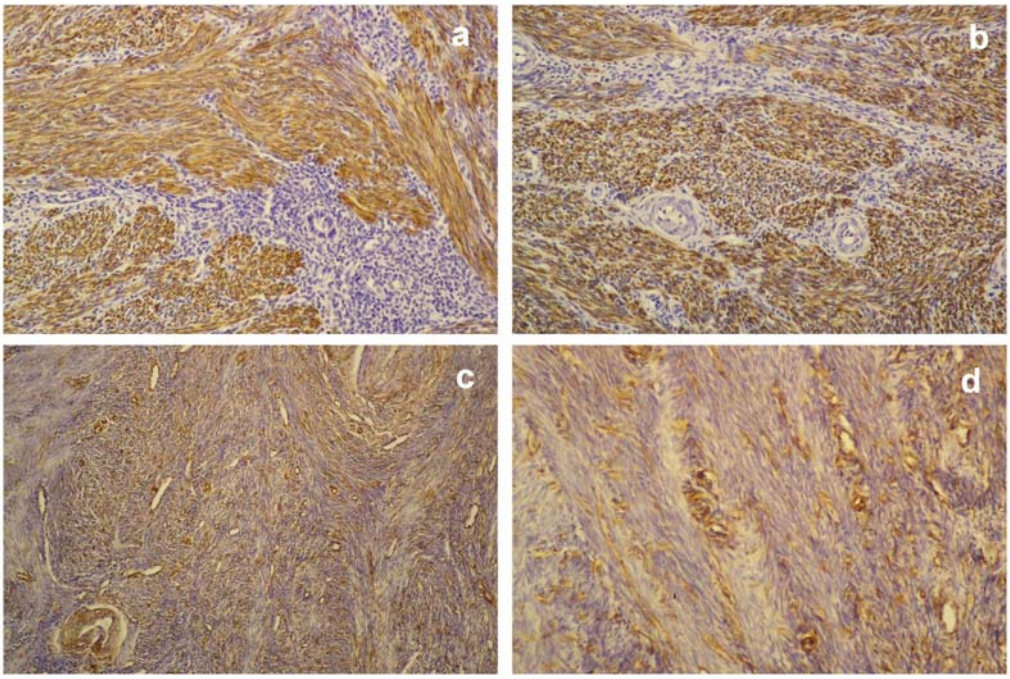
Rezultati

Na svim uzorcima submukoznih mioma uterusa analiziranih u ovoj studiji zapaženi su različiti stepeni atrofije endometrijuma iznad mioma, parenhim tumora sastavljen od glatkih mišićnih ćelija i vezivna stroma sastavljena od kolagenih i elastičnih vlakana, fibroblasta i krvnih sudova. Na analiziranim uzorcima nisu zapažene sekundarne promene, niti ulceracije endometrijuma. Prema histološkom tipu, 17 uzoraka klasifikovani su kao celularni miomi sastavljeni od gusto zbijenih glatkih mišićnih ćelija, dok je 8 uzoraka pripadalo vaskularnim miomima, sastavljenim od glatkih mišićnih ćelija između kojih su se nalazili krvni sudovi većeg ili manjeg prečnika.

Ispitivanjem fenotipskih karakteristika ćelija u sastavu analiziranih mioma uterusa utvrđeno je da najveći broj glatkih mišićnih ćelija u njihovom sastavu pokazuje imunoreaktivnost na α-glatkomišićni aktin i dezmin (slike 1 i 2). Mali broj glatkih mišićnih ćelija pokazivao je imunoreakti-



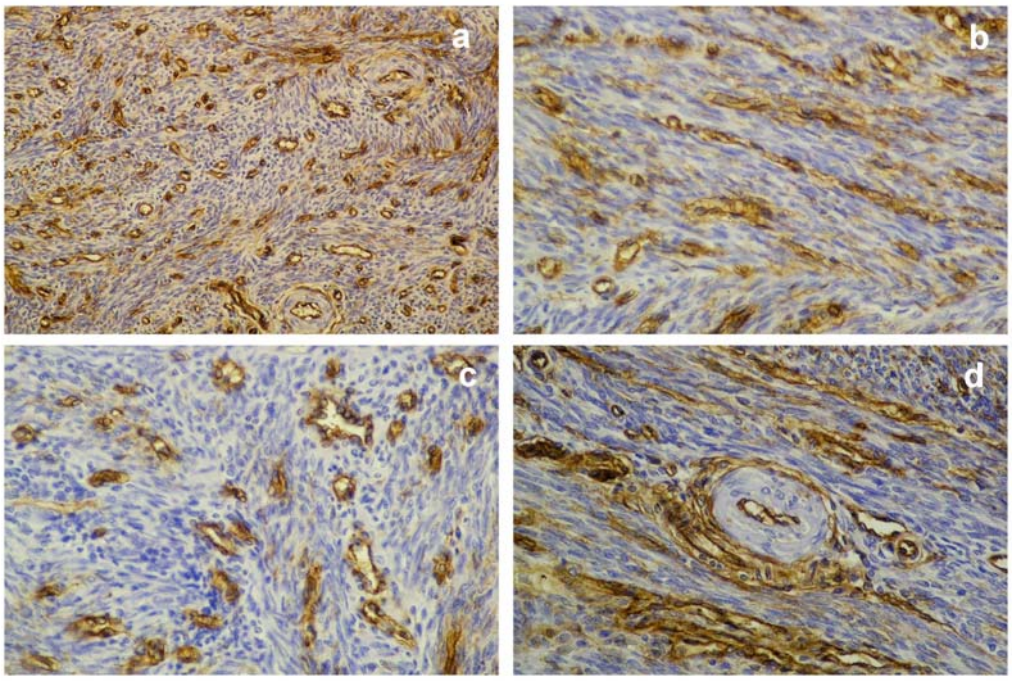
Sl. 1 – Glatke mišićne ćelije submukoznog mioma materice (imunocitohemijsko bojenje na α-glatkomišićni aktin)
a (× 32); b (× 64); c i d (× 128)



Sl. 2 – Glatke mišićne ćelije submukoznih mioma materice
a i b - imunohistohemijsko bojenje na dezmin, × 64
c - imunocitohemijsko bojenje na vimentin, × 32
d - imunocitohemijsko bojenje na vimentin, × 64

vnost na α-glatkomišićni aktin i vimentin (slike 1 i 2). Neke od vimentin-imunoreaktivnih glatkih mišićnih ćelija pokazivale su i imunoreaktivnost na PCNA. Ispitivanjem distribucije CD34 antigena, utvrđeno je njegovo prisustvo u fibroblastima vezivne strome, kao i u endotelu krvnih sudova mioma (slika 3). Osim ekspresije CD34 antigena, endotel krvnih sudova mioma, pokazivao je imunoreaktivnost na vimentin, dok su glatke mišićne ćelije u njihovom zidu pokazivale imunoreaktivnost na α-glatkomišićni aktin i dezmin (slike 1 i 2). Ispitivanjem distribucije CD45 antigena, na uzorcima je zapažena umerena ili slaba reakcija.

ce zbog prisustva vezivnotkivne komponente u miomima. Međutim, u svetlu eksperimentalnih radova koji datiraju od sredine dvadesetog veka, preovladalo je mišljenje da miomi, fibromiomi i fibromi materice, vode poreklo od glatkih mišićnih ćelija. Najranije studije, pokazale su u *in vitro* uslovima da miomi vode poreklo od glatkih mišićnih ćelija materice [13]. Međutim, glatke mišićne ćelije, pokazuju visokodiferentovan kontraktilni fenotip, dok je nastanak mioma povezan sa proliferativnom aktivnošću ćelija, koja je karakteristična za sintetski fenotip. Moguće je da vimentin-imunoreaktivne ćelije zapažene u našoj studiji, predstavljaju glatke mišićne ćelije



Sl. 3 a-d. – Submukozni miom materice (imunocitohemijsko bojenje na CD34, × 64)

Diskusija

Rezultati ove studije pokazali su da najveći broj glatkih mišićnih ćelija submukoznih mioma uterusa eksprimira α-glatkomišićni aktin i dezmin, dok mali broj ćelija eksprimira α-glatkomišićni aktin i vimentin. Prema podacima iz literature, glatke mišićne ćelije mogu da eksprimiraju kontraktilni ili sintetski fenotip. Migracija, proliferacija i sintetska aktivnost glatkih mišićnih ćelija određena je njihovim sintetskim fenotipom. Za razliku od glatkih mišićnih ćelija kontraktilnog fenotipa, ćelije sintetskog fenotipa nisu međusobno spojene komunikantnim spojevima. U citoplazmi imaju veoma razvijene sintetske organele, granulirani endoplazmatski retikulum i Goldžijev aparat, sa vrlo malo miofibrila. Ovaj fenotipski status je karakterističan za početne faze razvoja glatkih mišićnih ćelija, tokom embrionalnog života i tokom rasta. Ove nedovoljno diferentovane forme sintetišu kolagen, elastin i proteoglikane, pa podsećaju na fibroblaste. Odgovaraju na dejstvo citokina i specifičnih faktora rasta [12].

Dugo se smatralo da miomi materice vode poreklo od mišićnih ćelija ili od fibroblasta. Ova teorija imala je pristalisintetskog fenotipa, koje predstavljaju prekursore za nastanak mioma materice, što ćemo u nastavku razmotriti.

Posebno je zanimljivo prisustvo vimentin-imunoreaktivnih ćelija, zapaženih u ovoj studiji, koje su lokalizovane unutar traka glatkih miocita imunoreaktivnih na dezmin. Prema podacima iz dostupne literature, vimentin je protein molekulske mase 52–57 kDa u intermedijarnim filamenatima ćelija mezenhimalnog porekla (fibroblasta, endotelnih ćelija, glatkih mišićnih ćelija, hondrocita, limfocita i ćelija krvi), dok je dezmin proteinski molekul težine 53–55 kDa koji učestvuje u formiranju intermedijarnih filamenata diferenciranih glatkih, skeletnih i srčanih mišićnih ćelija [14]. Poznato je da glatke mišićne ćelije koeksprimiraju vimentin i dezmin. U patologiji uobičajeno se koristi imunohistohemijsko bojenje na vimentin za diferencijaciju malignih tumora mezanhimalnog porekla i karcinoma [15]. Međutim, i neki benigni tumori, pre svega bubrega, pluća, želuca, dojke, materice, jajnika i tireoidne žlezde, kao i mešoviti tumori pljuvačnih žlezda mogu da eksprimiraju ovaj antigen, što se smatra posledicom koekspresije vimentinskih i keratinskih filamenata u tumorskim ćelijama i činjenicom da antitela na vimentin mogu da

pokazuju ukrštenu reaktivnost sa citokeratinskim intermedijarnim filamentima [16]. Koekspresija vimentina i dezmina zapažena je i u nekim tumorima mekog tkiva kao što su rabdomiosarkomi i leiomiosarkomi [15].

Prema podacima iz literature, imunocitohemijsko dokazivanje prisustva glatkih mišićnih ćelija zasniva se na indirektnoj metodi dokazivanja prisustva α-glatkomišićnog aktina u sarkoplazmi ovih ćelija. U ranim mesecima, po rođenju, 50% glatkih mišićnih ćelija još uvek ne eksprimira α-glatkomišićni aktin, dok je takvih ćelija manje od 1% u odraslom organizmu. Pošto je α-glatkomišićni aktin dokazani marker diferencijacije glatkih mišićnih ćelija, očigledno je da se potpuna diferencijacija ovog fenotipa dovršava u mesecima nakon rođenja [17, 18]. Posle završene sinteze α-glatkomišićnog aktina, započinje ekpresija teških lanaca glatkomišićnog miozina i dezmina, pa se prisustvo ovih filamenata u sarkoplazmi može koristiti kao marker visokodiferenciranog kontraktilnog fenotipa [18]. Rezultati velikog broja savremenih istraživanja pokazali su da u nekim patološkim stanjima, poput vaskularnog remodelovanja, kongenitalnih oboljenja krvnih sudova, ali i tokom patogeneze tumora nastalih od mezoderma, glatke mišićne ćelije pokazuju izostanak reakcije na markere diferenciranog kontraktilnog fenotipa, odnosno na teške lance glatkomišićnog miozina i dezmin. Uporedo sa gubitkom dezminske ekspresije, dokazana je evidentna ekspresija vimentina, intermedijarnog filamenta koji je karakterističan za sve ćelije nastale od mezoderma. Pošto se ova dva intermedijarna filamenta, prema podacima iz literature, koeksprimiraju u glatkim mišićnim ćelijama, „pomeranje“ ekspresije ka vimentinu svedoči o gubitku kontraktilnih karakteristika i posledičnom nastanku sintetskog fenotipa [12, 17]. I druga istraživanja pokazala su da glatke mišićne ćelije i u zrelom dobu, u nekim različitim oboljenjima mogu da eksprimiraju sintetski fenotip. Smatra se da je jedan od ključnih momenata u patogenezi ovih oboljenja gubljenje ekspresije markera diferenciranog kontraktilnog fenotipa glatkih mišićnih ćelija, odnosno njihov prelazak iz kontraktilnog u sintetski fenotip. Ova fenotipska transformacija omogućava proliferaciju glatkih mišićnih ćelija i sintetsku aktivnost [16].

Rezultati studija drugih autora, pokazali su da se u sastavu mioma uterusa nalaze vretenaste glatke mišićne ćelije koje pokazuju koekspresiju vimentina i dezmina, dok veoma mali broj ćelija pokazuje samo imunoreaktivnost na vimentin, što je autorima ukazivalo na fibroblaste [10]. Transmisionom elektronskom mikroskopijom potvrđeno je prisustvo glatkih mišićnih ćelija diferenciranog kontraktilnog fenotipa i fibroblasta. Međutim, u pomenutoj literaturi nema podataka koji bi ukazivali na to da li je reč o miofibroblastima, odnosno glatkim mišićnim ćelijama sintetskog fenotipa, što bi bilo zanimljivo utvrditi u nekom daljem istraživanju.

Neke studije pokazale su da glatke mišićne ćelije mioma uterusa eksprimiraju dezmin i imaju morfologiju sličnu glatkim miocitima neizmenjenog uterusa, što sugeriše da se u sastavu ovih mioma nalaze glatke mišićne ćelije visokodiferentovanog kontraktilnog fenotipa [19]. Međutim, visokodiferencirane kontraktilne ćelije ne proliferišu, a jedan broj glatkih miocita analiziranih u našoj studiji, ali i u studijama drugih autora, pokazivao je proliferativnu aktivnost, odnosno PCNA-imunoreaktivnost.

Možda je fenotipska tipizacija prisutnih vimentin-imunoreaktivnih glatkih mišićnih ćelija zapaženih kako u našoj studiji, tako i u radovima drugih autora, razjašnjena u novijim podacima iz literature. Nedavno su, naime, u sastavu mioma uterusa identifikovane posebne vimentin-imunoreaktivne ćelije koje su lokalizovane između snopova glatkih mišićnih ćelija imunoreaktivnih na dezmin [20]. Ove ćelije razlikuju se prema svojim morfološkim karakteristikama od glatkih mišićnih ćelija i fibroblasta. Karakteriše ih okrugao ili ovalni oblik, bez citoplazmatskih nastavaka, pa su označene kao „poseban tip fibroblasta“ [21]. Utvrđeno je da ove ćelije odgovaraju na dejstvo inflamatornih citokina, da ih produkuju, da mogu da sintetišu prostaglandine i što je posebno bitno, da mogu da se diferenciraju u miofibroblaste, što ukazuje na njihovu slabu diferentovanost [22, 23]. Moguće je da su ove ćelije upravo prekursori ćelija mioma uterusa, odnosno, da predstavljaju neku vrstu progenitornih ćelija. I drugi radovi pokazali su da je reč o nedovoljno diferentovanim fibroblastima koji sintezom različitih aktivnih supstanci stvaraju uslove za razvoj tumora.

Međutim, naši rezultati pokazali su da vimentin-imunoreaktivne ćelije ne pokazuju imunoreaktivnost na CD34 što bi bila uobičajena karakteristika fibroblasta [24], tako da ostaje pretpostavka da je reč o nedovoljno diferentovanim glatkim mišićnim ćelijama sintetskog fenotipa. Nasuprot tome, fibroblasti u vezivnoj stromi pokazuju izrazitu CD34 imunoreaktivnost, uz izostanak reakcije na markere kontraktilnog fenotipa. Izrazitu CD34 imunoreaktivnost pokazuje i endotel velikog broja krvnih sudova prisutnih u vezivnoj stromi, međutim, njihova očuvanost lumena i jasna struktura zida uz prisutne atrofične promene endometrijuma materice sugerišu da nepravilna krvarenja kod žena sa submukoznim miomom materice nastaju kao posledica atrofičnog i istanjenog endometrijuma.

U submukoznim miomima materice analiziranim u ovoj studiji, bili su prisutni retki leukociti, odnosno, nije zapažena izražena inflamatorna reakcija.

Rezultati analize fenotipskih karakteristika ćelija submukoznih mioma materice analiziranih u ovoj studiji, mogli bi da budu u saglasnosti sa savremenim konceptom patogeneze mioma. Prema ovoj hipotezi, glatke mišićne ćelije materice vode poreklo od pluripotentnog uterusnog primordijuma, koji čine epitelne celomske i mezenhimne supcelomske ćelije. Razliku u nastanku endometrijuma i miometrijuma gotovo da je nemoguće odrediti, s obzirom na to da već u ranom embrionalnom dobu nestaje granica između epitela i mezenhima onih delova Muller-ovih kanala iz kojih nastaje uterus. Stoga, zbog biopotencijala ne može se jasno odrediti geneza neoplazmi koje se razvijaju u materici te su opravdane pretpostavke pojedinih autora da ćelije ovih tumora vode poreklo od stromalnih ćelija endometrijuma [23, 25].

Studije koje su izučavale razvoj glatkih mišićnih ćelija u tkivima koja vode poreklo od endoderma (digestivni i urinarni trakt) i od mezoderma (Miller) tokom fetalnog razvoja pokazuju da je razvoj glatkih mišićnih ćelija u mezodermal-

nim tkivima sporiji od razvoja glatkih mišićnih ćelija u endodermalnim tkivima. Nediferentovane ćelije koje proliferišu i diferenciraju se u glatke mišićne ćelije materice, tokom fetalnog perioda imaju duži period nestabilnosti pri čemu su izložene mnogobrojnim spoljašnjim faktorima kao što su steroidni hormoni ili faktori rasta majke. Nediferencirane ćelije na koje utiču, za sada, nepoznati faktori, verovatno postaju kasnije progenitorne ćelije od kojih se razvijaju miomi materice. Progenitorne ćelije ostaju u miomu i verovatno počinju rast posle menarhe, rastući tokom reproduktivnog perioda, kada postoji najveća aktivnost ovarijuma. U postmenopauzi, kada dolazi do pada koncentracije steroidnih hormona dolazi do prestanka rasta mioma materice [26].

Zaključak

Rezultati ove studije upućuju na zaključak da se u sastavu submukoznih mioma materice nalaze glatke mišićne ćelije visokodiferentovanog kontraktilnog fenotipa (imunoreaktivnost na α-SMA i dezmin), kao i glatke mišićne ćelije sintetskog fenotipa koje proliferišu (imunoreaktivnost na α-SMA i vimentin; imunoreaktivnost na PCNA). Za razliku od uobičajene strukture miometrijuma, u submukoznim miomima materice prisutna je velika količina vezivnog tkiva nastalog kao produkt sinteze fibroblasta koji se prema svojim imunohistohemijskim karakteristikama jasno razlikuju od glatkih mišićnih ćelija sintetskog fenotipa.

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Mechanical injuries of the eye: incidence, structure and possibilities for prevention

Mehaničke povrede oka: učestalost, struktura i mogućnost prevencije

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Abstract

Background/Aim. Despite technological advances used in everyday clinical practice, injuries of the eye caused by various agents still produce blindness and poor vision in a significant number of people. The aim of this study was to analyze factors leading to occurrence of mechanical injuries of the eye. **Methods.** Mechanic injuries of the eye in patients treated at the Institute for Eye Diseases of the Clinical Center of Serbia in Belgrade, in an eight-year period were analyzed. Investigated parameters were: sex and age of patients, their profession, time of injury (months, days and hours), place and way of injury and a visual acuity on admission and dismiss, as well as further follow-up. Type of injury (closed or opened injuries of the eyeball), with all the complications that followed were carefully noted and monitored. The time of primary surgical repair was noted and analyzed, whenever necessary. **Results.** In the period of eight years, 2 701 patients (2 257 males and 444 females) were treated in the hospital due to mechanical injury of the eye. Almost equally, both the right (50.5%) and the left eye (49.5%) were injured, while in 39 (1.4%) patients both eyes were injured at the same time. The injuries occurred in all age groups, but mostly in adults, employed persons, aged from 16 to 65 (70%). Among injured children, 18.8% were beyond the age of 15. Most frequent injuries occurred in workers (39%), and then in pupils (16.3%). Wood was the mean of injury in 23.7% of cases, sharp and pointed objects in 16.1%, hammering and metal particles in 14.4%, glass in 10.1%, and other different objects in the rest of 35.7% of all injured persons. There were other very serious means or mechanisms of eye injuries, like hair band, dog bite, rooster's beak, rubber bullet, etc. Considering months in the year and days in the week, the injuries were almost equally distributed, and related to the time of day even 75% occurred between 10 a.m. and 10 p.m. Most injuries (38.2%) occurred while doing some work out of professional working place, while only 25.4% injuries occurred at the working place. Most of the patients (30.3%) had visual acuity L+P+ (light perception with correct projection) only, on attendance, but it varied from complete blindness to 1.0. There were 1 282 blunt injuries (contusion) (47.5%) and 1 373 penetrating eyeball injuries (50.8%), while the rest (1.7%) were injuries of ocular adnexa. Most of the primary surgical treatments (63.7%) were done in the first 24 hours from the moment of the injury. At dismiss, visual acuity was normal in 53.2%, the eye was blind in 19.1% injured patients. **Conclusion.** The results of this study showed that the injuries occurred most frequently in actively working people and pupils, that men were injured five times more often than women; that wood, sharp objects and glass were the most common means, that there was an equal number of blunt injuries and penetrating wounds, and that it was very important to treat injury promptly, preferably within the first 24 hours. By further analysis, it might be concluded that many injuries could have been prevented, avoiding long medical treatment and accompanying costs, and what is most important - permanent invalidity caused by reduced visual function or blindness of the injured eye is avoidable.

Key words:
eye injuries; eye injuries, penetrating; incidence; diagnosis; treatment outcome; risk factors; risk management.

Apstrakt

Uvod/Cilj. Uprkos tehničkim dostignućima koja se koriste u svakodnevnoj kliničkoj praksi, mehaničke povrede oka izazvane različitim uzrocima još uvek dovode do slepila i smanjene sposobnosti vida kod značajnog broja ljudi. Cilj ovog rada bio je analiza faktora koji dovode do mehaničke povrede oka. **Metode.** Analizirane su mehaničke povrede oka kod bolesnika koji su bolnički lečeni u Institutu za očne bolesti Kliničkog centra Srbije u Beogradu u periodu od osam godina. Od parametara analizirani su pol i starost bolesnika, njihovo zanimanje, vreme kada se povreda desila (po mesecima, danima i satima), mesto i način gde su se povrede desile, kao i oštrina vida pri prijemu i pri otpustu ili kasnijim kontrolama. Analizirana je i vrsta povrede (zatvorena ili otvorena povreda očne jabučice) sa svim komplikacijama. Na kraju je dato i vreme kada je izvršena primarna hirurška obrada rane na oku, ukoliko je ona bila neop-

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hodna. **Rezultati.** U toku osam godina, zbog mehaničke povrede oka, bolnički je lečeno 2 701 bolesnik (2 257 osoba muškog i 444 osoba ženskog pola). Skoro podjednako bilo je povređivano i desno (50,5%) i levo oko (49,5%), dok su kod 39 (1,4%) bolesnika bila povređena oba oka istovremeno. Povrede su se dešavale u svim starosnim kategorijama, ali najčešće kod odraslih radno aktivnih osoba starosti od 16 do 65 godina (70,0%). Među decom uzrasta do 15 godina bilo je 18,8% povređenih. Najčešće su se povređivali radnici (39,0%), a zatim učenici (16,3%). Drvo je bilo uzrok povrede kod 23,7%, oštri i šiljati predmeti kod 16,1% ispitanika, čekić i metal kod 14,4%, staklo kod 10,1%, a drugi uzročnici kod preostalih 35,7% povređenih ispitanika. Bilo je i vrlo retkih uzročnika povrda oka, kao što su rajf, ujed psa, kljun petla, gumeni metak itd. Prema mesecima i danima, povrede su bile skoro podjednako raspoređene, a prema dobu dana, čak 75,0% povreda desilo se od 10 sati pre podne do 10 sati uveče. Najviše povreda (38,2%) desilo se pri obavljanju posla van profesionalnog radog mesta, dok je na poslu zadobijeno 25,4% povreda. Najveći broj bolesnika (30,3%) pri prijemu imao je vidnu oštrinu L+P+ (osećaj svetla sa tačnom projekcijom), ali je vidna oštrina na prijemu bila od amauroze do 1,0. Zabeležene su 1 282 kontuzione povrede (47,5%) i 1 373 penetrantne povrede očne jabučice (50,8%), dok su ostale (1,7%) bile povrede pomoćnih organa oka. Najveći broj primarnih obrada rane (63,7%) izvršen je u prva 24 časa od momenta povrede. Pri otpustu vidna oštrina je bila normalna kod 53,2%, dok je amauroza postojala kod 19,1% povređenih bolesnika. **Zaključak.** Rezultati ove studije pokazuju da se najčešće povređuje radno aktivno stanovništvo i učenici, (muškarci pet puta češće od žena), da su drvo, oštri predmeti i staklo najčešći uzročnici povreda, da je podjednak broj kontuzionih i penetrantnih povreda i da se neophodna primarna obrada rane preduzima najčešće u prva 24 časa od povrede. Daljom analizom ovih faktora može se zaključiti da su se mnoge povrede mogle prevenirati, čime bi se sprečilo dugotrajno lečenje i troškovi lečenja, a ono što je najvažnije, mogla se izbeći trajna invalidnost zbog smanjenja sposobnosti vida ili slepila na povređenom oku.

Ključne reči:
oko, povrede; oko, penetrantne povrede; incidenca; dijagnoza; lečenje, ishod; faktori rizika; rizik, kontrola.

Introduction

Eye injuries are an important cause of blindness and poor vision [1, 2]. They are more common in young people, causing permanent invalidity, as well as a reduced working and everyday life capability.

In children, injuries are one of the most common causes of blindness [3].

Eye injuries can be caused by various agents. In underdeveloped countries, as well as in rural areas, they are most frequently caused by wood [4], by branch or thorn, while in industrially developed countries they most frequently occur at place of work, sport grounds, or during recreation [5–7]. Children are more frequently injured at home or while playing, with blunt or sharp objects [8, 9] .

Although nowadays we do have powerful drugs and microsurgery reached unimagined limits, prognosis for serious eye injuries is still poor, in general [10, 11]. Eye injuries request long-lasting care, including hospital treatment, a long period of conservative medication, with a possibility of one or repeated surgeries [12, 13]. This has a big social and economic effects [11]. That is why in many studies, particularly in those dealing with epidemiology of eye injuries, the full attention has been given to preventive measures [6, 14–17].

In our previous paper [18] we dealt with similar problems of mechanical eye injuries, only for some shorter period of time - five years.

The aim of this study was to analyze factors leading to occurrence of mechanical eye injuries as well as to reveal the possibilities for prevention and, consequently, to reduce the number of eye injuries.

Methods

This prospective study included patients with mechanical injuries of the eye that had been treated at the Institute for Eye Diseases of the Clinical Center of Serbia in Belgrade during a period of eight years (January 1st 2000 - December 31st 2007). Only the patients with major traumas were hospitalized, and both contusions and penetrating injuries were analyzed.

The following factors were noted: sex and ages of patients, their occupation, place of injury, way of injury, time (concerning months, days and the time of the day), visual acuity in the moment of the first contact with the doctor, and visual acuity at dismiss, too. Types of injuries (blunt or penetrating) were noted, with all possible complications. In blunt eyeball injuries, damage to the structures in the eye were examined, as well as the eyeball rupture if present; in penetrating injuries, we studied the place of eyeball penetration, whether a scleral wound was in question, or both cornea and sclera were injured at the same time. Also, it was emphasized whether the injury was accompanied with a traumatic cataract or not. The number of penetrating injuries with a retained intrabulbar foreign body was shown. At the very end, we noted the time of primary surgical repair.

Results

As a result of mechanic injuries, in the period of eight years, 2 701 patients were hospitally treated. That means that an average of one patient a day was kept for treatment in the hospital. Of all hospitalized, there were in total 2 257 men (83.6%) and 444 women (16.4%), the male/female ratio being 5.1:1. Out of 2 701 patients, 39 (1.4%) had injuries to both eyes, while 2 662 (98.6%) had one eye injured. Of those, 1 364 (50.5%) had the right eye injured, 1 337 (49.5%) the left one. Injured patients aged from 2 to 88 years, with an average of 35.5 years of age (Figure 1).

Considering patient's occupation, there were 1 053 (39.0%) workers, 439 (16.3%) pupils, 309 (11.4%) unem-

ployed people, 240 (8.9%) farmers, 187 (6.9%) employees, 183 (6.8%) preschool children, 164 (6.1%) housewives, 60 (2,2%) students and 66 (2.4%) retired people injured (Figure 2).

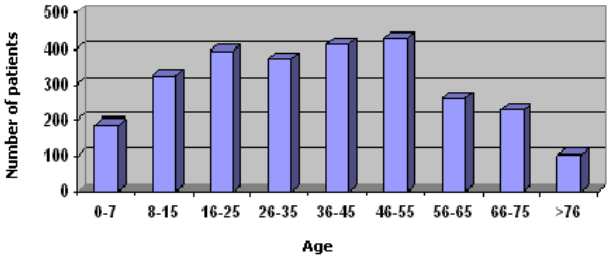

Fig. 1 – Age distribution of injured patients

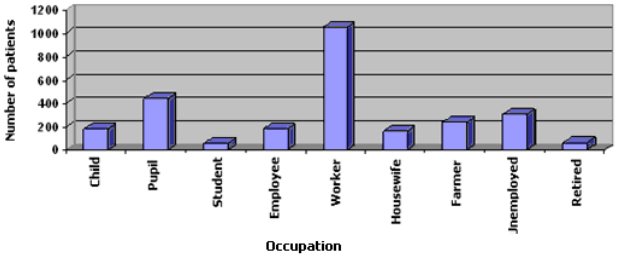

Fig. 2 – Distribution of injured patients according to their occupation

The causes and mechanisms of injuries were: wood in 639 (23.7%) studied persons, pointed and sharp objects in 435 (16.1%), hammering and metal particles in 389 (14.4%), glass in 274 (10.1%), cork of beer or any other bottle in 82 (3.0%), plastics in 119 (4.4%), electric battery explosion in 31 (1.1%), injuries due to falls in 150 (5.5%), eye injuries in fighting in 149 (5.5%), puck suspenders and elastic rubber bands in 64 (2.4%). The rest of the patients, 203 (7.5%), got injured in different, often bizarre manners as violations of the bale or an explasione object, for example (Figure 3).

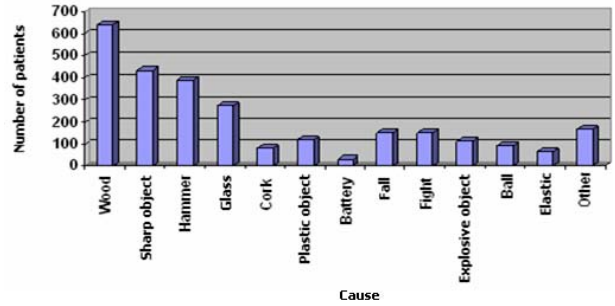

Fig. 3 – Distribution of injured patients according to cause and mechanism of injury

Injuries to both eyes occurred mainly in traffic accidents or were caused by explosive equipments. One psychotic patient was self-injured: with his hand, he pulled out his own right eyeball and destroyed the left one leading it to traumatic rupture and semievisceracion.

Month distribution was unremarkable (Figure 4). For the days in a week, the number of patients with eye injuries was also similar – varying from 299 on Tuesdays to 430 on Sundays (Figure 5).

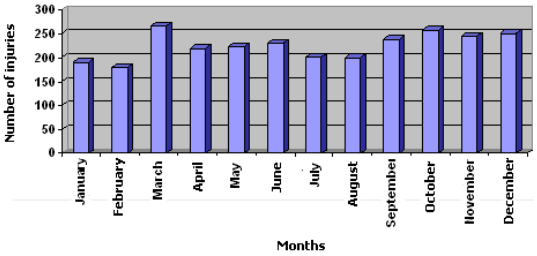

Fig. 4 – Number of injuries by months

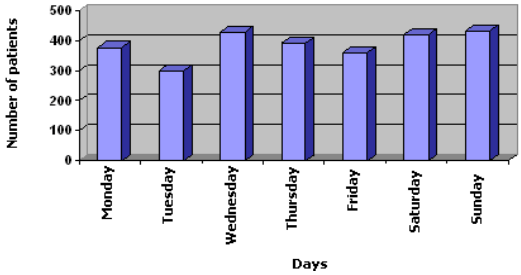

Fig. 5 – Number of patients with eye injuries by days in week

Taking a look at distribution of injuries depending on the part of a day, the day is divided into four-hour periods. Between 6 h a.m. and 10 h a.m. - 461 (17.1%) patients were injured, from 10 h to 14 h - 749 (27.7%), from 14 h to 18 h - 836 (31,0%), from 18 h to 22 h - 440 (16.3%), from 22 h to 02 h - 136 (5.0%), from 2 h to 6 h a.m. - 79 (2.9%) patients were injured (Figure 6).

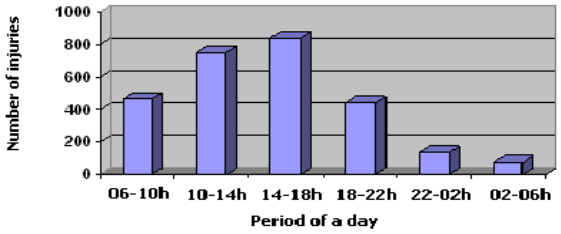

Fig. 6 – Number of injuries in different periods of a day

Injuries occurred in different places: at the professional working place in 686 (25.4%) patients, at work but not at the professional working place in 1031 (38.2%), at home in 336 (12.4%) patients, in traffic accident in 134 (5.0%), while playing in 217 (8.0%), during recreation in 130 (4.8%), at school in 106 (3.9%) patients (Figure 7).

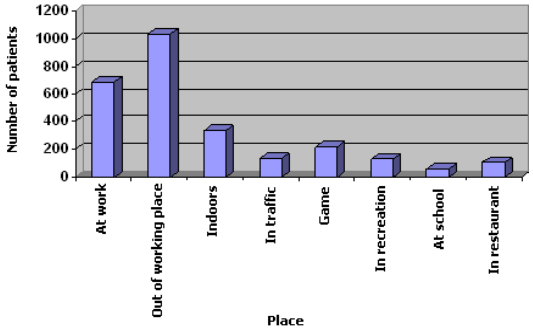

Fig. 7 – Distribution of injured patients depending on place of infliction of injuries

On admission, visual acuity in injured eye was: no light perception in 170 (6.2%), light perception without correct projection in 269 (9.8%), light perception with correct projection in 832 (30.3%), 1/60-0,1 in 466 (17.0%), 0.2-0.5 in 339 (12.4%), 0.6-0.8 in 254 (9.3%), and 0.9-1,0 in 410 (15%) (Figure 8).

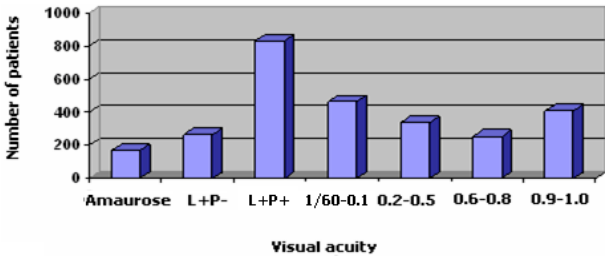

Fig. 8 – Distribution of injured patients according to visual acuity of the injured eye on admission
L - light perception, P – correct projection

Hospitalization of the patients with injuries of the eye lasted in average 6.7 days. Visual acuity upon dismiss had following values: no light perception in 441 (16.1%), light perception without correct projection in 112 (4.1%), light perception with correct projection in 151 (5.5%), from 1/60-0.1 in 103 (3.8%), from 02-0.5 in 140 (5.1%), 0.6-08 in 240 (8.8%), and 0.9 -1.0 in 1 514 (55.2%) patients (Figure 9).

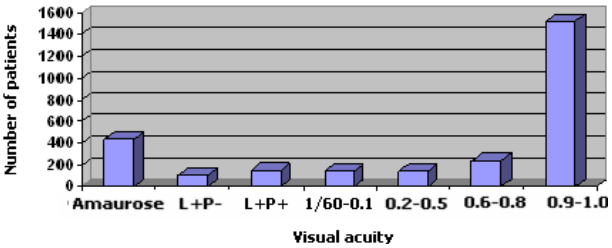

Fig. 9 – Distribution of injured patients according to visual acuity of the injured eye on dismiss
L - light perception, P – correct projection

This was not a definite visual acuity.

Blunt eyeball injuries occurred in 1 282 (47.5%), penetrating ones in 1 373 (50.8%) patients, while 85 (1.7%) were hospitalized due to an adnexal injury.

Hyphema was the most frequent cause of hospitalization in patients with blunt injuries. It was found in 1 123 (87.6%) cases, and in all of them it was the direct reason for hospitalization. Also we saw: iridodialysis in 62 (4.8%), iridorhexis in 33 (2.6%), subluxation of the lens in 146 (11.4%), lens luxation in 127 (9.9%), hemophthalmos in 74 (5.0%), edema and hemorrage of retina in 89 (6.9%), and the eyeball rupture in as much as 248 (19.30%) patients.

Total percentage of complications was more than 100%, as one injured eye sometimes harbored several complications. In 1 373 patients suffering a penetrating eyeball injury, the place of penetration was: cornea in 809 (58.9%), sclera in 265 (19.3%), and both cornea and sclera in 299 (21.8%) patients. The penetration through cornea was followed by traumatic cataract in 365 or 45.1% patients of all cases with perforated cornea, there was no cataract in the rest of 444 (54.9%) patients. Scleral perforation was accompanied with traumatic cataract in only 15 patients (5.7%). Perforation of both cornea and sclera with traumatic cataract was registered in 117 (39.1%), and without cataract in 182 (60.9%). Among patients with a penetrating injury of the eyeball, in 433 (31.5%) there was an intrabulbar foreign body of different nature.

Primary surgical repair was done in 1 522 patients. Operations were performed at different times after injury: within the first eight hours in 274 (18.0%) patients, in the period of 8 to 24 hours in 695 (45.7%), at the period of 24–48 hours in 260 (17.1%) and in more than 48 hours from the moment of injury in 293 (19.2%) patients (Figure 10).

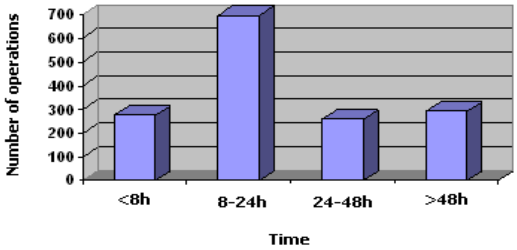

Fig. 10 – Number of operations according to the period between time of injury and primary surgical repair

Discussion

In one of our previous papers [18] we analyzed mechanical injuries of the eye with the same parameters for a period of five years. Now we carry out the same analysis again, but for a period of eight years with much more patients. Although the purpose of the study is the same as the previous one, our additional intention was to find out if anything changed in the meantime. This time, 2 701 patients with a total of 2 740 mechanical eye injuries were analyzed. This is a respectable group, so that the results and conclusions should be valid. We had an average of one serious hospitally treated injury each day in those 8 years. As the Institute for Eye Diseases of the Clinical Center of Serbia in Belgrade is a referent center for the treatment of eye injuries in Serbia, the majority of patients with serious eye injuries were treated there. Considering that Serbia has a population of approximately 8 million, then we see that the incidence of mechanical eye injuries is 4 out of 100 000 inhabitants per year. This number is somewhat bigger in fact, because a number of injured persons had been hospitalized and treated elsewhere: other university clinics or provincial eye departments. In various studies, the incidence of eye injuries per 100 000 inhabitants/year varies very much [19, 20].

Incidence of injury of the right and the left eye was almost equal. What particularly worries us, are the simultaneous injuries of both eyes, seen in 39 (1.4%) patients. Those were very serious cases, resulting in poor vision in both eyes, blindness in one and poor vision in other, or even blindness in both eyes.

In literature, there is a statement that eye injuries are more common in men than in women [21–24]. In our study,

there were 5.1 times more men than women. We discovered that the same was true in children and young, as well as in grown up persons, while in elderly - the number of injured males and females is almost equal. Such gender distribution is understandable when you know that boys play more dangerous games, also using toys that can injure the eye, while grown up men more frequently are exposed to eye injuries at work, in traffic, during recreation, in fight. Old persons, both men and women, are somewhat protected being generally less active. They are more frequently injured at home, moving around without enough care, or fall at home or in the street.

Anyhow, all age categories suffered injuries: small children, pupils, adults, working populations, but also retired and very old people. As it could be expected, among the injured were mostly working people, aged between 26 and 65 (54.4%). There has been a disturbing high percentage (6.8%) of injured children younger than 7 - preschool children. Among them, there were also children just one year old or slightly older, that were "accidentally" injured with a knife, glass or other sharp object which normally should not be found in the hands of such a small children. Dangerous objects or tools must not be kept in places reachable by children. Small children are not able to recognize the threat – the difference between dangerous and not dangerous objects - but parents or grandparents should always think about it. That is why we think that "by accident" should not exist: there is only our carelessness, our negligence and our incapability to foresee possible consequences of such a carelessness. If we look upon this problem seriously, almost all eye injuries could be avoided.

There was also found a considerable percentage of injuries in school children (12.0%), aged 8–15. They were injured in school (pencil, pair of compasses, chalk, glass), in the school yard (various games), at sport hours (ball, finger or elbow of a schoolmate), at home or outside. Again, in the majority of cases this was due to carelessness. Negligence is the primary cause of injuries. On the bases of a more complete analysis of causes and ways of getting injured, it was seen that such injuries could have been avoided, too, at least in 70% of the tested patients.

Also, in the biggest group of injured patients (actively working population) which we already mentioned, injuries are avoidable. Injuries at the working premises, in the first place, the injured person not using protective means prescribed by low. We also met a quite number of injured (38.2%) among those who were doing some other work, out of their professional place of work, for which they had not been prepared enough. There are two basic reasons for injuries. The first, not being trained enough for such kind of work, the second – work without any protection at all, although it was obligatory.

The retired were injured in different ways, most often at homes (fall in the bathtub, hitting the shower-battery, falling and hitting furniture edges), particularly at night. In the same group there were also elderly farmers, injured in stable, while working in field, cutting wood etc. – which only confirms what we already know that in villages old people still work hard. The prevention for those people is very much to be discussed, as injuries are always unexpected and do occur all of a sudden.

Analyzing occupational distribution of eye injuries, we see that the workers are most frequently injured (39.0% of all), industrial workers in particular, as well as car drivers. Many of them did not have any protection at all. They were also injured when not paying enough attention, not taking enough care. The same was true for drivers not using safety belts. A very high percentage of injured pupils had been registered, too. This group covers both the primary school and the high school pupils, to the age of 18, that is why the participation of the pupils among the tested injured persons is high (16.3%). This is really a very high percentage in the group of very serious injuries, which most frequently caused permanent disability, predetermining further life destiny of those young people. Keeping in mind all those parameters and already described ways of injury of younger school children, we must also be worried about the frequency of the injuries in older school children group (children attending high school), in fights, traffic, drinking. The importance of proper education of young people and their bringing up should be stressed, the family being always at the first place, followed by school and the whole society. That could contribute to prevention of a considerable number of injuries. University students were seldom injured (2.2%), for many reasons. It is tre that they represent the smallest group of all, spending most of their time studying in the libraries, not having so much possibilities to be injured.

The employed, with one eye injury (6.9%), represent a surprisingly high percentage if we take into consideration their occupations. However, they were not injured at work, but working at some other places instead, during recreation, in car accidents, etc. The prevention measures taken during all these activities could certainly decrease the number of injuries in persons working in offices. The injured housewives (6.1% of all), usually get injured at home, in the garden, or in the field. This is the least protected group among injured persons, hardly having been protected at. Farmers were injured while working in the field, or any other place. For this group of injured, endophthalmitis following penetrating eyeball injury was characteristic, being more common than in other groups. We can hardly talk about prevention at work, but the fact is that they contact the doctor much later, sometimes even several days after the injury, when serious complications already occurred. They usually believe that the injury is not serious, that it will be healed all by itself, sometimes claiming that visiting a general practitioner in order to be referred to an ophthalmologist is a loss of time.

Only a small number of unemployed persons (2.4%) had an eye injury. They had been injured either dealing with something themselves, or by standing close to other persons watching them working or helping them.

The injuries occurred in various ways and were provoked by various causes. The most frequent cause (23.7%) was piecing wood (cutting wood with an axe). Those were usually serious blunt injuries followed by hyphema, lens luxation into the vitreous or anterior chamber, iridodialysis, but still more often by rupture of the eyeball and prolapse of

the interior eye structures. Such injuries, in most instances, ended in amaurosis. It is interesting that both men and women were equally injured by a piece of wood in that way. We are dealing with wood also in those injuries caused by a branch of a tree when cutting it, walking or running in the woods, or processing wood in a circle-cutter.

Although as a rarity, this kind of injury was also seen in a pilot of agricultural aircraft, flying low with an open clam and consequently hitting a branch of tree and resulting in a scleral injury. Injury caused by tree branch is most frequently seen in farmers or women who cultivate flowers. Injury by a rod, or a wooden stick we usually met in children playing outside; such injuries used to be very serious, regardless whether it was only a contusion or an eyeball penetration.

The second group classified by frequency (16.1%) represented injuries caused by pointed or sharp objects: knife, fork, wire, armature, a piece of grinding panel, and similar. Those were always penetrating injuries of the eyeball with no retained intraocular foreign body. Both children and adults were injured in that way.

Hammering of various objects or polishing metal, were the third common cause of eye injuries (14.4%). When hammering, a piece of metal could penetrate into the eyeball (penetrating injuries of the eyeball with the retained foreign body). According to the patient's explanations, it was not rare that such an injury was caused by hammering a stone, sawing a tree, etc. Most of the patients were aware of having got a piece of stone or wood into the eye. However, such injuries were almost always caused by a piece of metal from a hammer or an axe. Seldom, a perforation of the eyeball occurred with a foreign body retained in the orbit.

Broken glass was also the frequent cause of eye injuries (10.1%). In car accidents, broken windshields caused irregular, star-shaped corneal or scleral wounds. In other causes wound edges were more regular and good for surgery. Much to our surprise, there was quit a number of injuries caused by glass of a bottle broken out or in the refrigerator. All the patients stated that the battles exploded spontaneously, without any physical effort or damage. It is possible that the sudden change of temperature was the cause, or may be an inproper pressure of the contents, because it was always a full battle that exploded. In such cases, the producer's responsibility should be always questioned.

Injuries caused by beer or mineral water bottle cork were classified in a special group (3.0%). In a short time period, we had numerous eye injuries caused by bullets from a children plastic gun toy. Those were always blunt injuries with hyphema, usually with subluxation or luxation of the lens, with iris damage and retinal edema. Children were injured, not those holding a gun, but those standing next to them, most frequently - girls. Those injuries could have been avoided if the parents have been warned in time about the possibility of such accidents, before buying such a dangerous toy.

Injuries obtained in fight were found in 5.5% of all injured persons. They were usually caused by the fist, or any object that the attacker had in the hand (boxer, broken bottle, stick, gun handle). Depending on this, the injuries were contusions or penetrating, accompanied by adnexal damage.

Injuries that occurred when falling (5.5%) were seen both in children and adults, particularly in elderly persons. The most common place of accident in children was school. The adults were usually injured outside, when in a hurry they stumbled and fell, hitting the eye against any extended object. Injuries often occurred at home. In the bathtub, when the patient slept and hit the shower battery. Usually, such injuries caused rupture of the eyeball and visual loss, but frequently loss of integrity of the eye, too. The later was also due to fall in the flat, hitting the edge of the furniture. This most often happened to elderly people, when walking in the night with no light on. Certainly, some of those injuries could have been prevented - slip in the bathtub is avoidable if a special plastic rag had been placed, and the light should always be on while walking at night.

Injuries caused by explosive devices most frequently occurred due to explosion of firecrackers and other similar pyrotechnical objects. We have seen bomb detonators, bombs and fire weapons in that group of explosion injuries, too. Injuries caused by firecrackers and other pyrotechnical objects usually occur at time of mass celebrations, particularly the New Year's Eve. They are usually very serious, with a lot of foreign body particles in the cornea inside the eye itself, in the case of penetrating injuries of the eyeball, always with serious loss of function. Children are the usual victims, but the adults, too. Those injuries could have been prevented if legal prohibition of sale of dangerous and potentially harmful toys was followed. Parents should have a special task: not to buy or not to permit their children to buy such toys, in order to avoid serious self-injuries in their children or doing harm to other people.

Injuries by ball occurred in young boys, mostly during recreation, but in professional sportsmen, too. Usually such injuries occurred while playing football, basketball and handball and there were always eyeball contusion with hyphaema.

Injuries caused by electric battery explosion were not common (1.1%), but they usually were very serious and, beside the mechanic effect, there were all signs of a caustic burn caused by sulfuric acid.

Injuries caused by various elastic rubber bands occurred in 2.2% of all cases, but they were extremely serious, too.

Some patients experienced injuries by cow horn, stone or other object coming out of a mower. There were also some bizarre, but serious injuries, like self-injury, injuries caused by bee bite, cock pickling, piece of a disk (for disk record), rubber bullet from a police fire-weapon, etc.

We also analyzed the time of injury, in order to check whether there were some months in a year, or days in a week, or particular moments of a day, when injuries occurred more often, in order to suggest special measures of prevention to be taken at that specific period, if any. We found that injuries were generally almost equally distributed by months in a year (the differences not being statistically significant) (Figure 4). Similar distribution was found when days in a week were checked: injuries equally occurred both on working days and during weekends (Figure 5). However, incidence of injuries varied depending on the part of day. Be-

tween 6 h and 10 h a.m., the incidence was low (17.1%). Usually, those were workers injured at work, or farmers or housewives who started with their work early. In the period between 10 h and 14 h, the number of injuries was much higher (27.7%). At that period of time all sorts of injuries occurred. The highest incidence (31%) was in the afternoon (14 h–18 h), when all sorts of already mentioned injuries occurred. People are tired by the end of a working day and there are more children in the streets, playing. When we know all this, it becomes clear that we should be particularly careful at this period of the day. In the evening (18 h –22 h), the number of injuries somewhat decreases, while incidence in the night (22 h–06 h) was considerably lower compared to other periods of the day (Figure 6), which is understandable, as the most of people seep in the night. Patients, who had been injured then, were either fighting in restaurants, were drunk, or had car accidents. We also met injuries at work during the night shifts, but they were few.

Analysis of the places where injuries had occurred is very interesting (Figure 7). One should expect most injuries to occur at work, which some investigators stated in their studies. However, we found a lower percentage of injuries at work (25.4%), compared to those that happened somewhere else (38.2%). One of the reasons could be that this study was carried on at the time of economic crisis in our country, with many unemployed people, who, in order to survive, had to work very often wherever possible. Consequently, many jobs had been done by unqualified persons or without normal protection, injuries therefore being more frequent. Injuries that occurred at home or in yards were seen in 12.4%. At home, children were injured while playing with sharp and pointed objects, but the same was true for the adults, particularly housewives injured at home. Home injuries were caused by knife or broken glass, were due to falls in the bathtub, falls in the room hitting the edges of furniture, etc., while yard injuries were caused by wood, branch of a tree or a thorn. We should think of those possibilities in time, as many of them are avoidable. Some people were injured by a soccer ball, tennis ball or a racket, some other had a contact with a branch of tree while walking or jogging in parks or woods. Children were injured playing. Those injuries were caused by stick, rubber band or a hand. At school, 3,9% of all injuries occurred. Another 5% were those that occurred in car accidents. They perhaps do not look very high, but two thing are very remarkable: firstly, almost always a very young or a younger person was injured, secondly: both eyes were injured too often simultaneously. Restaurants are typically the place where injuries do occur in fights. It is usually at night, and in most instances people are drunk. Those injuries are caused by fists, glass or broken battles, or any other object that was at hand. Drunk people were often injured upon fall.

Out of a total of 2 701 patients that were hospitalized for an eye injury, in 1 282 of them (47.5%) there was a contusion of the eye. In many of those patients (87.6%), there was a hyphema, which was the main reason for hospitalization. Hyphema ranged from the first degree to a total one. Beside hyphema, some patients had also damages to other structures of the eyeball: iridodialysis, iridorrhexis, subluxation or luxation of the lens, hemophthalmos, retinal edema or hemorrhagies, rupture of the choroids. A considerable number of complete eyeball ruptures (248 or 19.3% of the total number of blunt injuries) worried us particularly. Those contusions followed by rupture of the eyeball, were usually caused by a hit with a piece of wood and a cow horn, or occurred upon fall, hitting an exposed objects. Usually there was a prolapse of eyeball contents, usually ending in amaurosis. The second half of the mechanic traumas treated in the hospital (1 373 cases or 50.1%) were penetrating eye injuries. Penetration through the cornea was the most common (58.9%), more than half of those patients had also a traumatic cataract. Patients with corneal perforation without cataract were treated with less difficulties. In one fifth of the patients (19.3%) we met a scleral wound. The next one, fifth of the patients (21.8%) had a perforation of both cornea and sclera simultaneously, the wound spreading across the limbus. Those were serious injuries. In most of the cases with corneal perforation there was a vitreous prolapse, too, and with both corneal and scleral perforation – a prolapse of uveal tissues and the vitreous. Of all penetrating injuries of the eyeball, in 433 (31.5%), there was an intraocular foreign body retained. The nature of foreign bodies was different: most frequently that was iron, but also copper, bronze, wood, plastics, glass.

Beside those two groups of patients, with blunt and with penetrating eyeball injuries, there was a small group of 46 (1,7%) patients without injuries of the eyeball itself. Serious injuries of the ocular adnexa (eyelids and lacrimal drenage system, in most of the cases) was the reason for hospitalization of those people.

The visual acuity on admission ranged from amaurosis to normal vision. It was not measured in small children or in adults when it was not possible due to objective reasons. In 6.2% of patients, injured eye was blind on admission, in another 9.8% there was a perception of light without correct projection, in 30.3% on admission there was a light perception with correct projection. Such a visual acuity had nod discouraged us, but we tried to preserve the integrity of the eyeball at the moment of the primary treatment, while with further reconstruction later on we did our best to improve the visual function. In 53.7% of patients the visual acuity was between 1/60 and 1.0 on admission (Figure 8).

Conservative treatment and surgery resulted in a much better visual function on dismiss. It ranged from amaurosis to normal vision (Figure 8). Time of the primary treatment of the wound is essential. The best would be if it was done within the first eight hours after injury, but we did our best to have it done in the first 24 hours. Our opinion is that surgical repair of a seriously injured should not be performed by a doctor on duty – at night. We preferred to treat such injuries with the help of experienced surgeons or a team of ophthalmologists, first thing next morning. We had 18.0% of patients operated in the first eight hours, which is unsatisfactory and that percent should be higher. We had 45.7% patients operated between 8 h and 24 h after injury, which is acceptable, but we must try to improve that which could be done by better organization. In 17.1% of patients, the period from injury and the operation was between 24 h and 48 h,

which is not satisfactory, as well as the percentage of 19.2% patients who were operated even later, several days after the accident. Such late surgery was usually done in patients from the country province, that reached the clinic later. The result of late surgical repairs were much worse.

Conclusion

The results of this study showed that the injuries occurred most frequently in actively working people and pupils, that men were injured five times more often than women; that wood, sharp objects and glass were the most common means, that there was an equal number of blunt injuries and penetrating wounds, and that it was very important to treat injury promptly, preferably within the first 24 hours.

It might be concluded also that prevention is necessary and that it should be our major task in future. By prevention, many of typical injuries could be avoided, as well as the consequences resulting in poor vision or blindness, the same being true for economic costs for treatment, rehabilitation and absence from work. Prevention is possible at any age, any place and in all activities mentioned.

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Analiza stepena ezofagitisa, hromendoskopskog i histološkog nalaza jednjaka kod ispitanika sa gastroezofagusnom refluksnom bolešću pre i posle terapije

Analysis of the grade of esophagitis, chromendoscopical and histological findings of esophagus in patients with gastroesophageal reflux disease before and after the therapy

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Apstrakt

Uvod/Cilj. Simptomi gastroezofagusne refluksne bolesti (GERB) među najčešćim su tegobama zbog kojih se bolesnici upućuju gastroenterologu. Nastaje kao rezultat dejstva refluksiranog želudačnog sadržaja koji ide u jednjak. Prevalencija svih oblika GERB iznosi 40%. Cilj ovog rada bio je analiza stepena ezofagitisa, hromendoskopskog i histološkog nalaza jednjaka kod ispitanika sa GERB, pre i posle terapije. **Metode.** Prospektivnom studijom bilo je obuhvaćeno 90 bolesnika sa simptomima GERB koji su bili podeljeni u dve grupe u zavisnosti od toga da li imaju endoskopske znakove gastroezofagusnog refluksa (grupa ERB), ili su znaci refluksa odsutni (grupa NERB). Kod svih ispitanika urađena je ezofagogastroduodenoskopija, hromoendoskopsko bojenje, test na *Helicobacter pylori* i histološki nalaz jednjaka. Kod ispitanika pozitivnih na *Helicobacter pylori* sprovedena je eradikaciona terapija. **Rezultati.** Ezofagitis stepena B bio je zastupljen kod najvećeg broja ispitanika. Približno isti broj ispitanika imao je pozitivan nalaz hromoendoskopije u obe grupe. Posle terapije kod obe grupe ispitanika hromoendoskopija bila je statistički značajnije negativna nego hromoendoskopija pre terapije (p = 0,00001). Umnožavanje, elongacija papila, hiperplazija bazalnih ćelija, vaskularna dilatacija, povećanje mitotske aktivnosti, prisustvo polimorfonuklearnih leukocita statistički je bio češći histološki nalaz u grupi ERB nego grupi NERB. Posle terapije, u obe grupe ispitanika statistički ređi bio je histološki nalaz odgovarajućih parametara jednjaka. **Zaključak.** Hromoendoskopija u kombinaciji sa standardnom endoskopijom povećava senzitivnost i specifičnost za refluksnu bolest. Histologija u refluksnoj bolesti povezana je sa endoskopskom i kliničkom slikom, tako da lokalizacija uzimanja biopsija i histološki kriterijumi patohistoloških promena moraju biti jasno definisani. Umnožavanje i elongacija papila, hiperplazija bazalnih ćelija i prisustvo polimorfonuklearnih leukocita najrelevantniji su kriterijumi za postavljanje dijagnoze NERB.

Ključne reči:
gastroezofagusni refluks; ezofagitis; endoskopija; histologija; helicobacter pylori; osetljivost i specifičnost.

Abstract

Background/Aim. The symptoms of gastroesophageal reflux desease (GERD) are among the most common complaints for which patients are indicated for visiting gastroenterologist. It occurs as a result of the effect made by gastric reflux contents that moves into the esophagus. The prevalence of all forms of GERD is 40%. The aim of this study was to analyze the grade of esophagitis, chromendoscopical and histological findings of esophagus in patients with GERD before and after the therapy. **Methods.** A prospective study included 90 patients with symptoms of GERD, divided into 2 groups depending on whether they had endoscopic signs of gastroezophageal reflux (group ERD), or not (group NERD). All the patients had esophagogastroduodenoscopy, chromoendoscopy staining, test for *Helicobacter pylori* and histological findings of the esophagus. In the patients with *Helicobacter pylori* infection eradication therapy was done. **Results.** Esophagitis-B level was present in most of the patients. Among the groups, roughly the same number responded to positive findings on chromoendoscopy. After the therapy, chromoendoscopy was significantly negative in both groups of the patients comparing to chromoendoscopy before the therapy (p = 0.00001). Multiplication and elongation

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of papilla, basal cell hyperplasia, vascular dilatation, increasing of mitotic activity and the presence of polymorphonuclear leukocyte cells were statistically more frequent histological findings in the group ERB compared to the group NERB. After the therapy, the patients in both groups had statistically less histological findings of appropriate esophageal parameters. **Conclusion.** Chromoendoscopy combined with the standard endoscopy increases the sensitivity and specificity for reflux disease. Histology in the reflux disease is associated with endoscopic and clinical findings so that the localization of taking biopsies and histological criteria of pathohistological changes must be clearly defined. Multiplication and elongation of papilla, basal cell hyperplasia and the presence of polymorphonuclear leukocytes are the most relevant criteria in the diagnosis NERD.

Key words:
gastroesophageal reflux; esophagitis; endoscopy; histology; helicobacter pylori; sensitivity and specificity.

Uvod

Simptomi gastroezofagusne refluksne bolesti (GERB) među najčešćim su tegobama zbog kojih se bolesnici upućuju gastroenterologu. Kod 20% populacije simptomi bolesti traju duže od jedne nedelje [1]. Termin GERB koristi se da opiše simptome i promene sluznice jednjaka koje nastaju kao rezultat dejstva refluksiranog želudačnog sadržaja na jednjak [2]. Prevalencija svih oblika GERB iznosi 40% [1, 3]. Termini peptički ezofagitis, refluksni ezofagitis i erozivni ezofagitis, erozivna refluksna bolest (ERB) su sinonimi za podgrupu bolesnika sa GERB kod kojih se patohistološki dokazuju promene mukoze jednjaka koje obično korelišu sa simptomima refluksa kiselog sadržaja [4]. Ozbiljnost epitelnih promena procenjuje se prisustvom simptoma ili endoskopskih znakova. Većina bolesnika sa GERB klinički se manifestuje blagim ili umerenim stepenom bolesti. Poslednjih godina u gastroenterološkoj literaturi pominje se termin neerozivne refluksne bolesti – NERB za bolesnike sa simptomatskim GERB koji nemaju makroskopske promene sluznice tokom proksimalne endoskopije. Procenjuje se da 50–70% bolesnika sa GERB ima NERB [5, 6].

Gastroezofagusni refluks manifestuje se kada postoji prolazni pad tonusa donjeg ezofagusnog sfinktera, što omogućava da želudačni sadržaj prodire u jednjak [1–3]. Male količine refluksa hlorovodonične kiseline (HCl) ispoljavaju se fiziološki, ali ako je produženo vreme kada je pH < 4, gastroezofagusni refluks smatra se patološkim. Produženo izlaganje jednjaka HCl i digestivnim enzimima koji se nalaze u želudačnom sadržaju ili duodenalnog sadržaja koji regurgitira u želudac (žučne soli) može da indukuje iritaciju sluznice jednjaka i da rezultira simptomima i morfološkim promenama sluznice jednjaka [4].

Simptomi i znaci refluksne bolesti jednjaka mogu biti različitog intenziteta, nisu uvek u korelaciji sa težinom oštećenja sluznice jednjaka [4], i mogu biti porekla iz digestivnog trakta ili ekstraezofagusni [5]. Barrettov jednjak i razvoj adenokarcinoma jednjaka najznačajnija su komplikacija GERB [7], pa je cilj prevencije adenokarcinoma jednjaka, koji se sprovodi širom sveta, adekvatna terapija inhibitorima protonske pumpe [8]. Savremenom dijagnostikom najrazličitijim metodama treba otkriti razvoj metaplastičnih i displastičnih promena i primeniti adekvatnu terapiju.

Kada je dijagnoza GERB sumnjiva, endoskopija treba da bude prva metoda za utvrđivanje prisustva ezofagitisa i komplikacija hroničnog oštećenja jednjaka kiselinom, kao i da isključi druge uzroke bolesnikovih tegoba. Endoskopija je neophodna u proceni svih bolesnika sa hroničnim refluksnim simptomima. Inicijalna endoskopija pomaže da se odredi stadijum bolesti. Spektar endoskopskih promena kod bolesnika sa GERB varira od makroskopski normalne sluzonice u donjem delu jednjaka kod oko 50% bolesnika (endoskopski negativni nalaz ili NERB), do nalaza erozija, ulkusa, strikutra i Barrett-ovog jednjaka (endoskopski pozitivan ili ERB) [9].

U novije vreme kod bolesnika sa GERB u toku endoskopije primenjuje se rastvor Lugola koji se raspršuje na sluznicu (hromoendoskopija). Rastvor lugol sastoji se od miksture joda i kalijum jodida, koji pokazuje afinitet za glikogen u nekeratinizujućem skvamoznom epitelu i primenjuje se u koncentracijama od 0,5 do 5%. Normalni epitel jednjaka bogat je glikogenom i prebojiće se crno, tamnobraon ili zeleno-braon bojom ubrzo nakon aplikacije, postepeno bledeći od minuta do jednog sata. Mukozne promene – displastične i neoplastične promene, inflamirani skvamozni epitel koji se vide u erozivnom ezofagitisu i neskvamozni epitel, kao što je cilindrični epitel, imaju malu količinu, ako uopšte i imaju glikogena, pa se neće prebojiti nakon aplikacije Lugolovog rastvora [10]. Bojenje Lugolom može olakšati dijagnozu karcinoma jednjaka u ranoj fazi i poboljšati preoperativnu procenu i širenje [11]. Pre bojenja senzitivnost i specifičnost za identifikaciju visokostepene displazije ili invazivnog karcinoma, na osnovu vidljivih endoskopskih promena bila je 62% i 79%, ponaosob. Nakon bojenja, neobojene površine identifikuju ove promene sa senzitivnošću od 96% i specifičnošću od 63%. Međutim, 23% bolesnika sa ozbiljnim displastičnim promenama i 55% bolesnika sa umerenim displastičnim promenama otkriveni su samo nakon bojenja Lugolom. Bojenje prema Lugolu poboljšava dijagnostiku Barrettovog jednjaka. Povećanjem demarkacije između pločastoslojevitog i cilindričnog epitela, povećava se senzitivnost, specifičnost i tačnost endoskopske dijagnoze Barrettovog jednjaka na 89%, 93% i 91%, ponaosob [12]. Ova tehnika koristi se i za pokazivanje površina rezidualne Barrettove mukoze nakon različitih endoskopskih ablativnih tehnika [13].

Cilj rada bio je da se analizira hromendoskopski nalaz kod ispitanika grupa ERB i NERB, pre i posle terapije, da se analizira stepen ezofagitisa pre i posle terapije i da se uporede histološki nalazi jednjaka, pre i posle terapije.

Metode

Prospektivnom studijom u Klinici za gastroenterologiju Kliničkog centra u Nišu bilo je obuhvaćeno 90 bolesnika sa simptomima gastroezofagusne refluksne bolesti. Bolesnici su

bili podeljeni u 2 grupe u zavisnosti od toga da li imaju endoskopske znakove gastroezofagusnog refluksa (ERB grupa), ili su znaci refluksa odsutni (NERB grupa).

U istraživanju su bile korišćene sledeće metode: prikupljanje anamnestičkih podataka i klinički pregled ispitanika, ezofagogastroduodenoskopija sa biopsijom, histološki pregled sluznice i hromoendoskopsko bojenje. Tokom proksimalne endoskopije makroskopski je procenjivana sluznica jednjaka, želuca i duodenuma, uzimane su po dve biopsije antruma i korpusa, dve biopsije kardije, kao i četiri biopsije jednjaka na udaljenosti od 2 cm od Z-linije. U bioptatima sluznice želuca ispitivano je prisustvo *Helicobacter pylori* (*H. pylori*) infekcije, kao i histomorfološke promene sluznice, metodama hematoksilin eozin i metodom modifikovane Giemsae. Kod bolesnika sa *H. pylori* infekcijom sprovedena je eradikaciona terapija u trajanju od 7 dana inhibitorima protonske pumpe sa antimikrobnom terapijom (terapija I ili terapija II reda).

U bioptatima sluzokože jednjaka procenjivan je broj papila, elongacija papila, hiperplazija bazalnih ćelija, vaskularna dilatacija, prisustvo balonirnih ćelija, odsustvo sazrevanja ćelija, povećanje mitotske aktivnosti i polimorfonuklearni leukociti (neutrofili/eozinofili) [14]. Ezofagusna mukozna biopsija teoretski je korisna kod bolesnika sa simptomima GERB koji imaju normalni izgled sluznice tokom ezofagogastroduodenoskopije. Senzitivnost i specifičnost biopsije značajno varira u različitim studijama [15]. Kod svih bolesnika urađena je hromoendoskopija 2%-tnim rastvorom Lugola uz uzimanje biopsija sa neobojene sluznice. Stepen ezofagitisa je određivan prema klasifikaciji Los Angeles [16].

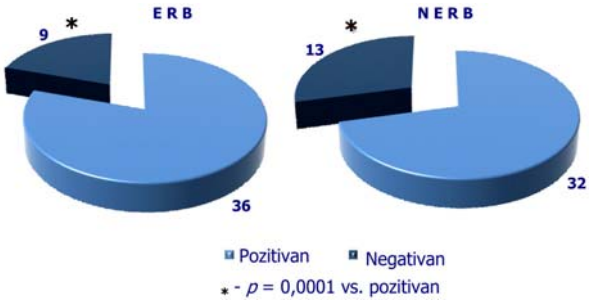
Nakon sprovedene eradikacione terapije i lečenja refluksne bolesti osam nedelja inhibitorima protonske pumpe, uz prekid unošenja inhibitora protonske pumpe od dve nedelje i blokatora H2 receptora, kao i ukidanje antibiotske terapije od najmanje četiri nedelje, ponovljena je biopsija sluznice kardije i jednjaka na već opisani način.

Podaci su obrađivani korišćenjem standardnih deskriptivnih statističkih metoda (srednja vrednost, standardna devijacija i procentualna zastupljenost). U radu je primenjivano više vrsta testova: Studentov *t*-test za uparene i neuparene uzorke i χ^2 test. Obrada dobijenih podataka izvršena je korišćenjem statističkog programskog paketa – *Statistical Package for Social Science* (SPSS) softverom, verzija 11.0 u Windows okruženju.

Rezultati

Prosečna starost ispitanika grupe sa ERB bila je 62,55 godina, a u grupi ispitanika sa NERB 58,15 godina. Prosečna starost ispitanika u obe grupe nije se bitnije razlikovala. Zastupljenost ispitanika različitog pola bila je približno ista u grupi ERB – muškarci 53,33%, žene 46,66%. U grupi ispitanika NERB bilo je više muškaraca (62,22%), nego žena (37,77%). Svi ispitanici imali su simptom gorušice. Gorušica je bila podjednako zastupljena u grupama u odnosu na doba dana. Kod obe grupe ispitanika gorušica se češće javljala danju (ERB, $p = 0{,}00001$; NERB, $p = 0{,}00001$), dok je manji broj ispitanika iz obe grupe imao gorušicu noću. Simptom regurgitacije češće se javljao kod ispitanika grupe ERB kod 28 ispitanika u odnosu na 21 ispitanika grupe NERB, ali bez statističke značajnosti.

Ezofagitis stepena B bio je zastupljen kod najvećeg broja ispitanika, kod 64,44%, stepena A kod 26,66%, dok je stepen C ezofagitisa bio zastupljen kod 8,88 %. Najveći broj ispitanika iz grupe ERB imao je ezofagitis stepena B. Ezofagitis stepena D nije nađen ni kod jednog ispitanika. Hromoendoskopija bila je pozitivna kod 36 ispitanika grupe ERB, dok je pozitivan nalaz zabeležen kod 32 ispitanika grupe NERB. Statistički značajno češće pozitivna hromoendoskopija bila je u grupi ERB ($\chi^2 = 32{,}4$; $p = 0{,}0001$), kao i u grupi NERB ($\chi^2 = 16{,}04$; $p = 0{,}0001$) u odnosu na negativan nalaz hromoendoskopije u okviru iste grupe. Između grupa nije bilo razlike u pozitivnom nalazu hromoendoskopije (slika 1).



Sl. 1 – Hromoendoskopski nalaz kod ispitanika sa endoskopskim znacima gastroezofagusnog refluksa (ERB) i bez endoskopskih znakova gastroezofagusnog refluksa (NERB)

Eradikaciona terapija sprovedena je kod 23 ispitanika grupe ERB i 31 ispitanika grupe NERB koji su bili *H. pylori* pozitivni. Posle terapije u grupi ispitanika sa ERB statistički značajnije ređi bio je endoskopski nalaz ezofagitisa ($\chi^2 = 22{,}26$; $p = 0{,}00001$) (tabela 1). Posle terapije u grupi ispitanika sa NERB, tri ispitanika imala su endoskopski nalaz ezofagitisa. Kod obe grupe ispitanika posle terapije, hromoendoskopija bila je statistički značajno češće negativna u odnosu na hromoendoskopiju pre terapije ($\chi^2 = 19{,}7$; $p = 0{,}00001$) (tabela 2).

Umnožavanje papila ($p = 0{,}0001$), elongacija papila ($p = 0{,}002$), hiperplazija bazalnih ćelija ($p = 0{,}0001$), vasku-

Tabela 1

Prisustvo ezofagitisa nakon terapije kod ispitanika grupa ERB i NERB

Vreme ispitivanja	ERB (n = 23)		NERB (n = 31)	
	Ezofagitis (n/%)	Uredan nalaz (n/%)	Ezofagitis (n/%)	Uredan nalaz (n/%)
Pre terapije	23 / 100	0	0	31 / 100
Posle terapije	8 / 34,78	15 / 65,22	3 / 9,67	28 / 90,33
χ^2 (*p*)	22,26 (0,00001)			

ERB – ispitanici sa endoskopskim znacima gastroezofagusnog refluksa; NERB – ispitanici bez endoskpskih znakova gastroezofagusnog refluksa

Tabela 2

Hromoendoskopija nakon terapije kod ispitanika grupa ERB i NERB

Vreme ispitivanja	ERB (n = 23)		NERB (n = 31)	
	Pozitivna (n/%)	Negativna (n/%)	Pozitivna (n/%)	Negativna (n/%)
Pre terapije	20 / 86,96	3 / 13,04	26 / 83,87	5 / 16,13
Posle terapije	5 / 21,73	18 / 78,26	3 / 9,68	28 / 90,32
χ^2 (p)	19,7 (0,00001)		34,27 (0,00001)	

ERB – ispitanici sa endoskopskim znacima gastroezofagusnog refluksa; NERB – ispitanici bez endoskpskih znakova gastroezofagusnog refluksa

larna dilatacija (p = 0,002), povećanje mitotske aktivnosti (p = 0,0001), prisustvo polimorfonuklearnih leukocita (p = 0,026) statistički značajni bio je češći histološki nalaz u grupi ERB nego u grupi NERB (p = 0,0001). Prisustvo baloniranih ćelija i odsustvo sazrevanja ćelija češće se javljalo kod ispitanika grupe ERB nego grupe NERB, ali bez statističke značajnosti (tabela 3). Posle eradikacione terapije, u grupi ispitanika sa ERB statistički značajno ređi bio je histološki nalaz umnoženih papila (p = 0,00001), elongiranih papila (p = 0,001), hiperplazije bazalnih ćelija (p = 0,00001), vaskularne dilatacije (p = 0,00001), baloniranih ćelija (p = 0,00002), odsustva sazrevanja ćelija (p = 0,03), povećanje mitotske aktivnosti (p = 0,00003) kao i prisustvo polimorfonuklearnih leukocita (p = 0,0001). Posle eradikacione terapije, u grupi ispitanika sa NERB, takođe, bio je ređi histološki nalaz umnoženih i elongiranih papila, ali bez statističke značajnosti. U grupi ispitanika sa NERB statistički značajno ređi bio je histološki nalaz umnoženih papila (p = 0,0009), hiperplazija bazalnih ćelija (p = 0,001), vaskularne dilatacije (p = 0,002), baloniranih ćelija (p = 0,002), povećanje mitotske akltivnosti (p = 0,03) i prisustvo polimorfonuklearnih leukocita (p = 0,00001) (tabela 4).

Tabela 3

Poređenje histoloških promena jednjaka između ispitanika grupa ERB i NERB

Histološke promene jednjaka	ERB (n = 45)		NERB (n = 45)		χ^2 (p)
	da	ne	da	ne	
Umnožene papile	45	0	33	12	13,85 (0,0001)
Elongacija papila	36	9	22	23	9,50 (0,002)
Hiperplazija bazalnih ćelija	39	6	17	28	14,7 (0,0001)
Vaskularna dilatacija	21	23	8	37	9,08 (0,002)
Prisustvo baloniranih ćelija	18	27	11	34	–
Odsustvo sazrevanja ćelija	8	37	4	41	–
Povećanje mitotske aktivnosti	30	15	11	34	16,17 (0,0001)
Polimorfonuklearni leukociti	44	1	38	7	4,94 (0,026)

ERB – ispitanici sa endoskopskim znacima gastroezofagusnog refluksa; NERB – ispitanici bez endoskpskih znakova gastroezofagusnog refluksa

Tabela 4

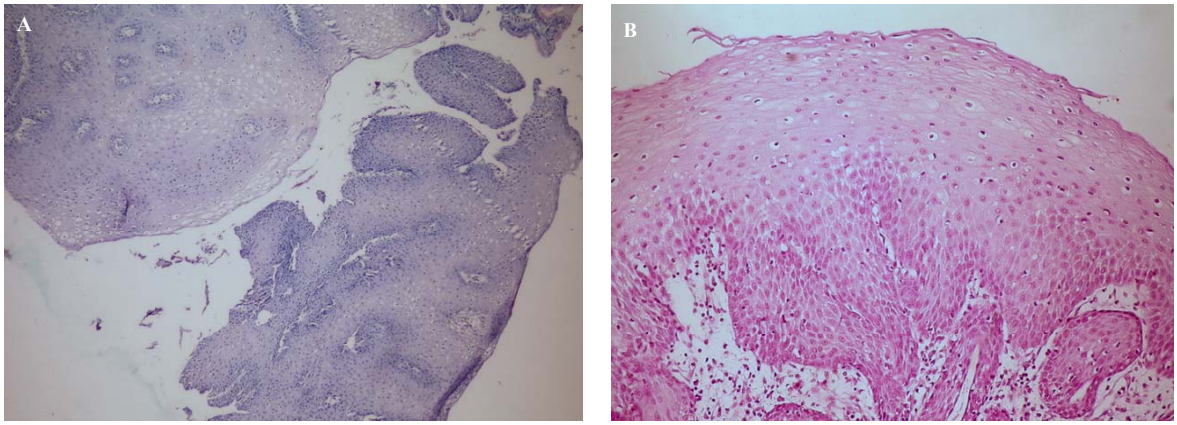
Poređenje histoloških promena jednjaka između ispitanika grupa ERB i NERB pre i posle terapije

Histološke promene jednjaka	ERB (n = 23)		NERB (n = 31)	
	Da	Ne	Da	Ne
Umnožene papile – pre terapije	23	0	22	9
Umnožene papile – posle terapije	9	14	9	22
χ^2 (p)	20,13 (0,0001)		10,9 (0,0009)	
Elongacija papila – pre terapije	22	1	17	14
Elongacija papila – posle terapije	13	10	8	13
χ^2 (p)	9,68 (0,001)		–	
Hiperplazija bazalnih ćelija – pre terapije	22	1	14	17
Hiperplazija bazalnih ćelija – posle terapije	6	17	3	28
χ^2 (p)	23,37 (0,00001)		9,8 (0,001)	
Vaskularna dilatacija – pre terapije	14	7	8	23
Vaskularna dilatacija – posle terapije	0	23	0	31
χ^2 (p)	22,5 (0,00001)		9,2 (0,002)	
Prisustvo baloniranih ćelija – pre terapije	13	10	8	23
Prisustvo baloniranih ćelija – posle terapije	0	23	0	31
χ^2 (p)	18,12 (0,00002)		9,19 (0,002)	
Odsustvo sazrevanja ćelija – pre terapije	4	19	0	31
Odsustvo sazrevanja ćelija – posle terapije	0	23	0	31
χ^2 (p)	4,38 (0,03)		–	
Povećanje mitotske aktivnosti – pre terapije	18	5	4	27
Povećanje mitotske aktivnosti – posle terapije	4	19	0	31
χ^2 (p)	17,08 (0,00003)		4,28 (0,03)	
Polimorfonuklearni leukociti – pre terapije	23	0	27	4
Polimorfonuklearni leukociti – posle terapije	12	11	5	26
χ^2 (p)	14,46 (0,0001)		31,26 (0,0001)	

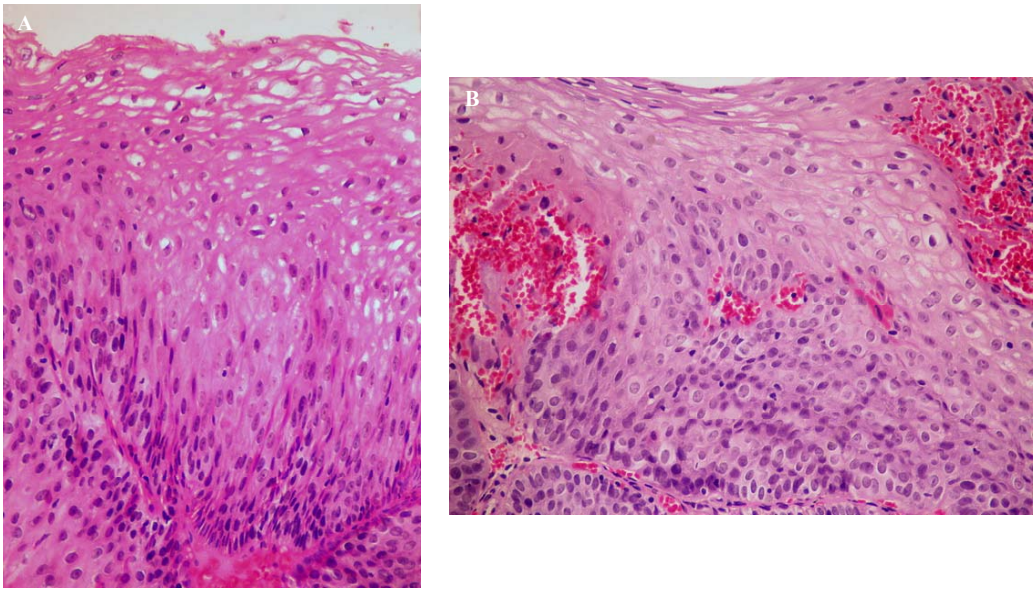
ERB – ispitanici sa endoskopskim znacima gastroezofagusnog refluksa; NERB – ispitanici bez endoskpskih znakova gastroezofagusnog refluksa

Pokazano je da se glikogenska akantoza ne može koristiti kao dijagnostički kriterijum za refluksnu bolest (slike 2–4).

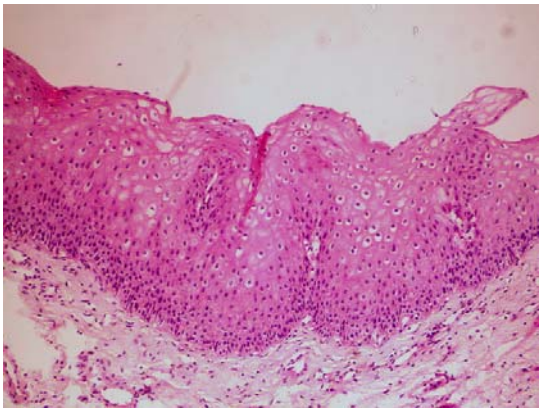
približno ista u grupi ERB, dok je u grupi ispitanika NERB bilo više muškaraca nego žena. Kada je u pitanju uticaj soci-



Sl. 2 – Pločastoslojeviti epitel sa akantozom i papilomatozom – A) HE, ×4; B) HE, ×20



Sl. 3 – Hiperplazija bazalnog sloja pločastoslojevitog epitela jednjaka, sa leukocitima A) HE, ×10; B) HE, ×20



Sl. 4 – Balonizovane ćelije u pločastoslojevitom epitelu (HE, ×10)

Diskusija

Prosečni uzrast ispitanika kod obe grupe nije se bitnije razlikovao. Zastupljenost ispitanika različitog pola bila je

odemografskih faktora na refluksne simptome, pol ne čini značajan faktor. Studije udruženosti godina i refluksnih simptoma dale su nekonzistentne rezultate. U jednoj britanskoj studiji prevalencija refluksnih simptoma rasla je sa godinama

starosti, ali je opadala nakon 70. godine života [17]. Nilsson i sar. [18] saopštili su istovetni trend samo za muškarce, ali ne i za žene. Saopštena je i veća prevalencija refluksnih simptoma kod osoba sa nižim socioekonomskim statusom.

Svi ispitanici imali su simptome gorušice, uglavnom danju. Prevalencija najčešćih simptoma GERB, gorušice i regurgitacije je visoka u opštoj populaciji sa učestalošću od 26%–61,2% [17]. Dnevne simptome imalo je 6% populacije, 14–20% jednom nedeljno, dok mesečne simptome ima 44% populacije SAD [19]. U studiji Nocon i sar. [20] 14% ispitanika imalo je umerene, a 4–5% ozbiljne simptome. Regionalne razlike saopštila je i studija Digest sa tromesečnom stopom prevalencije gorušice od 5% u Švajcarskoj u poređenju sa 22% u SAD [21].

Hromoendoskopija bila je statistički značajno pozitivnija u grupi ERB, kao i u grupi NERB u odnosu na negativan nalaz hromoendoskopije u okviru iste grupe. Najveći broj ispitanika iz obe grupe imao je pozitivan nalaz hromoendoskopije, što govori u prilog prednosti hromoendoskopije u odnosu na standarnu endoskopiju. Nakon terapije, kod obe grupe ispitanika hromoendoskopija bila je statistički značajnije češće negativna u odnosu na hromoendoskopiju pre terapije. Negativan nalaz hromoendoskopije pokazuje da u distalnom delu jednjaka nema glandularnog epitela. Hromoendoskopija je metoda kojom se postiže detaljna analiza mukozne površine, što uključuje površinsku vaskularnu arhitekturu koja predstavlja rani znak maligne transformacije, analizu površnih glandularnih otvora i nalaz makroskopskog tipa lezija. Hromoendoskopijom postiže se izvaredna demarkacija prekanceroznih promena i potreban je manji broj biopsijskih uzoraka uzetih nasumice, mada se u pojedinim studijama ne navodi prednost ovih metoda u odnosu na konvencionalnu endoskopiju [22]. Nekoliko studija pokazuju da su polja koja su ostala neobojena nakon prskanja Lugolovim rastvorom povezana sa displazijom i karcinomom [23]. Ova tehnika, takođe, identifikuje skvamoglandularnu spojnicu i omogućava bolje uzimanje biopsija cilindričnog epitela. Takođe, ova metoda pomaže u identifikaciji zaostalih Barrettovih ostrvaca nakon ablacione terapije [24, 25].

U poslednje tri decenije opisani su histološki kriterijumi refluksne bolesti, a to su: bazalna ćelijska proliferacija, regenerativna elongacija i porast broja epitelnih papila, porast broja i ektazija kapilara u epitelnim papilama, glikogenska akantoza u pločastoslojevitom epitelu, prisustvo tzv. „*ballon cells*“ (kiselinom izazvane degenerativne promene u pločastoslojevitom epitelu) i dilatacija intraćelijskih prostora u parabazalnim delovima pločastoslojevitog epitela. Kiselina oštećuje pločastoslojeviti epitel i to oštećenje praćeno je regenerativnim promenama, prvenstveno u zadebljanju bazalnog ćelijskog sloja (proliferativna zona) i elongaciji epitelnih papila sa ektazijom kapilara unutar papila [26]. Pomoću visokoosetljivih endoskopa pojedini kapilari mogu se prepoznati kao male crvene tačkice i viđaju se kod bolesnika sa refluksnom bolešću [27, 28]. Intaepitelni inflamatorni infiltrat čine: neutrofili, eozinofili i intraepitelni limfociti. Infiltracija neutrofilima po definiciji uvek je znak akutne inflamacije [26]. Neutrofili se nikada ne nalaze u normalnom skvamoznom epitelu, prisutni su na granici blizu erozije ili ulceracije. Neutrofili se mogu naći samo u nekim formama refluksne bolesti, ali ne mogu biti korišćeni u dijagnozi endoskopski negativne refluksne bolesti. Veruje se da je prisustvo eozinofila visokosenzitivno u histološkoj dijagnozi refluksne bolesti. Verovatno su nespecifični znak inflamacije koji nije uvek neophodno prisutan kod svih bolesnika [29]. Veruje se da je prisustvo T-limfocita normalni nalaz kod skvamoznog epitela. Retko se zapažaju kod bolesnika sa NERB i mogu biti prisutni u kontrolnoj grupi kao normalan nalaz [30]. U kombinaciji sa drugim markerima mogu pomoći u određivanju težine refluksne bolesti. Dilatacija intercelularnih prostora može biti pouzdani marker refluksne bolesti. Nađena je u visokoj prevalenciji kod bolesnika sa NERB i, takođe, pokazuje odgovor na acido- supresivnu terapiju [31]. Ove studije bazirane su na elektronskoj mikroskopiji, koja daje odgovor na pitanje kada je oštećenje počelo i kada je ezofagitis izlečen. Dilatacija intercelularnih prostora nije prazan prostor, već je ispunjen različitim proteinima koji omogućavaju ćelijski kontakt [32]. Neki izveštaji ukazuju da „*balloon cells*“ mogu biti znak veoma rane promene u refluksnom oštećenju. Jedna grupa autora pokazala je da postoji korelacija između glikogenske akantoze i refluksne bolesti. S obzirom na to da postoji samo jedan rad o tome, glikogenska akantoza se ne može razmatrati kao dovoljan marker za refluksnu bolest i ne može se koristiti kao dijagnostički kriterijum za refluksnu bolest [33].

Većina studija koristi kriterijume koje su preporučili Ismail-Beigi i sar. [34], a koji su bazirani na uzimanju biopsija iz distalnog ezofagusa na 2 cm iznad Z-linije. Uzimajuću u obzir dosadašnje studije, postaje jasno da je histologija pouzdana metoda u detekciji mikroskopskih inflamacija i regenerativnih promena kod bolesnika sa GERB i da ima važnu ulogu u dijagnozi. Neki autori zaključuju da dilatacija intercelularnih prostora može biti rani znak (ako je biopsija uzeta po standardizovanoj metodi) sa dobrom senzitivnošću i specifičnošću za refluksnu bolest, posebno ako je analizirana i dužina papila i debljina bazalne ćelijske hiperplazije. Histološka klasifikacija mora biti udružena sa kliničkim nalazom kroz pažljivu povezanost histoloških i kliničkih nalaza u refluksnoj bolesti [32].

Zaključak

Hromoendoskopija u kombinaciji sa standardnom endoskopijom povećava senzitivnost i specifičnost za postavljanje dijagnoze refluksne bolesti. Histologija u refluksnoj bolesti povezana je sa endoskopskom i kliničkom slikom, tako da lokalizacija uzimanja biopsija i histološki kriterijumi patohistoloških promena moraju biti jasno definisani. Dilatacija intracelularnih prostora, elongacija papila, hiperplazija bazalnih ćelija i prisustvo polimorfonukleranih leukocita najrelevantniji su kriterijumi za postavljanje dijagnoze NERB. Gastroezofagusna refluksna bolest je multifakorijalna bolest i zahteva zauzimanje kritičnog stava prema etiološkim faktorima.

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Living unrelated donor kidney transplantation – a fourteen-year experience

Transplantacija bubrega od živog nesrodnog davaoca – 14 godina iskustva

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Abstract

Background. In countries without a national organization for retrieval and distribution of organs of the deceased donors, problem of organ shortage is still not resolved. In order to increase the number of kidney transplantations we started with the program of living unrelated – spousal donors. The aim of this study was to compare treatment outcome and renal graft function in patients receiving the graft from spousal and those receiving ghe graft from living related donors. **Method.** We retrospectively identified 14 patients who received renal allograft from spousal donors between 1996 and 2009 (group I). The control group consisted of 14 patients who got graft from related donor retrieved from the database and matched than with respect to sex, age, kidney disease, immunological and viral pretransplant status, the initial method of the end stage renal disease treatment and ABO compatibility. In the follow-up period of 41 ± 38 months we recorded immunosuppressive therapy, surgical complications, episodes of acute rejection, CMV infection and graft function, assessed by serum creatinine levels at the beginning and in the end of the follow-up period. All patients had pretransplant negative cross-match. In ABO incompatible patients pretransplant isoagglutinine titer was zero. **Results.** The patients with a spousal donor had worse HLA matching. There were no significant differences between the groups in surgical, infective, immunological complications and graft function. Two patients from the group I returned to hemodialysis after 82 and 22 months due to serious comorbidities. **Conclusion.** In spite of the worse HLA matching, graft survival and function of renal grafts from spousal donors were as good as those retrieved from related donors.

Key words:
kidney transplantation; tissue donors; treatment outcome; transplantants; graft survival; graft rejection; risk assessment.

Apstrakt

Uvod/Cilj. U zemljama koje namaju nacionalnu organizaciju za prikupljanje i distribuciju organa od umrlih osoba, nedostatak organa još uvek je nerešen problem. Program transplantacije bubrega od živog nesrodnog davaoca – supružnika započet je da bismo povećali broj transplantacija. Cilj ispitivanja bio je da se uporedi preživljavanje bolesnika, grafta i funkcija grafta kod primalaca kojima su davaoci nesrodnici – supružnici sa onima kojima su davaoci bili živi srodnici. **Metode.** Retrospe-

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ktivnom analizom identifikovali smo 14 bolesnika koji su dobili bubreg od supružnika u periodu od 1996. do 2009. godine (grupa I). U kontrolnoj grupi bilo je 14 bolesnika koji su bubreg dobili od živog srodnog davaoca. Odabrani su iz postojeće baze uparivanjem sa bolesnicima grupe I prema polu, životnoj dobi, osnovnom bubrežnom oboljenju, imunološkom i virusološkom pretransplantacionom statusu, prvom modalitetu lečenja terminalne bubrežne slabosti i ABO kompatibilnosti. U periodu praćenja, 41 ± 38 meseci, registrovani su imunosupresivna terapija, hiruške komplikacije, epizode akutnog odbacivanja, infekcija citomegalovirusom i funkcija grafta preko nivoa serumskog kreatinina na početku i kraju praćenja. Svi bolesnici su pretransplantaciono imali negativanu unakrsnu reakciju. Pretransplantacioni titar ABO izoaglutinina kod krvno-grupno nepodudarnih bolesnika iznosio je nula. **Rezultati.** Bolesnici sa supružničkim graftom imali su značajno lošiju HLA podudarnost ($p < 0,01$). Nisu uočene razlike među grupama u praćenim parametrima. Dva bolesnika iz prve grupe vratila su se na hemodijalizu nakon 82, odnosno 22 meseca zbog teških komorbiditeta. **Zaključak.** Uprkos lošijoj HLA podudarnosti, supružnički graftovi imali su podjednako dobro preživljavanje i funkciju kao i bubrezi dobijeni od srodnog davaoca.

Ključne reči:
transplantacija bubrega; tkivo, davaoci; lečenje, ishod; graftovi; graft, preživljavanje; graft, odbacivanje; rizik, procena.

Introduction

In spite of tremendous breakthrough in the field of organ transplantation, organ shortage remains as the main problem [1]. Two main sources of organs are living related and deceased donors, with better graft function and survival in the former group [2–8]. In countries like Serbia, without a national organization for retrieval and distribution of organs of the deceased, problem of organ shortage is much more pronounced. In attempt to increase the number of kidney transplantations we initiated high-risk transplant programs across positive cross-match, incompatible blood groups and from living unrelated – spousal donors. With these new transplant programs the total number of kidney transplantations in our unit increased by 15%. Transplantations from living unrelated – spousal donors contribute with 5%. The later situation is extremely delicate, because two emotionally and economically related members of the same family and their children are at possible risk at the same time [9]. The aim of this study was to investigate if there was any difference in the patients' and renal graft survival and function between recipients whose donors were unrelated – spouses compared to those whose donors were related.

Methods

This study was a retrospective matched pairs analysis of two groups. Both groups of kidney allograft recipients from spousal (the group I) and related donors (the control group) consisted of 14 patients transplanted in the period from 1996 to 2009. In 13 patients from the group I and 11 from the control group primary kidney disease was discovered in the advanced or end stage renal failure (ESRF) with laboratory and clinical presentation suggestive of chronic glomerulonephritis. One patient in each group had renal polycystic disease. They had bilateral nephrectomy prior to transplantation. One patient in the control group had IgA nephropathy and one lupus nephritis. The initial treatment of ESRF in 12 patients in the group I and 11 in the control group was in a period of one to four years hemodialysis, two patients in each group had preemptive kidney transplantation and one patient from the control group was one year on continuous ambulatory peritoneal dialysis (CAPD). Two patients from the group I and one from the control group had positive anti-hepatitis C virus (HCV) antibodies, while one from the group I was hepatitis B surface antigen (HBs Ag) and hepatitis B e antigen (HBe Ag) positive. The human leukocyte antigen system (HLA) matching between recipients and donors in the group I was from 10% to 62.5% (mean 39.2 ± 15%), and in the control group from 50% to 63% (mean 50.8 ± 3.3%). All the patients who got ABO compatible graft had negative pretransplant cross-match (one from the group I who had historically positive cross-match). The recipients of ABO incompatible grafts had pretransplant isoagglutinine titer zero and negative cross-match [10–12].

The spousal donors aged from 33 to 60 years, mean 42.5 ± 7.5 years, and the related donors were significantly older, aged from 50 to 74 years, mean 60.14 ± 8.43 years ($p < 0.01$).

A potential donor and recipient were presented to the Ethical Committee of our institution if no exclusion criteria for transplant were met. After being accepted for the transplant program, the pretransplant evaluation was completed, and the couple was presented to the surgical team, which made a final decision about the operation.

All the patients got triple immunosuppressive protocol consisting of steroids, an antiproliferative drug (azathioprine, mycophenolate mofetil or mycophenolate sodium) and a calcineurine inhibitor (cyclosporine A or tacrolimus). Steroids were slowly tapered according to a standard protocol. The dose of antiproliferative drug was standardized, but also decreased during the follow-up. The calcineurine inhibitors' dose was adjusted depending on C0, C2 concentration and mini area under the curve of the first 4h (AUC 0–4). One patient with positive pretransplant cross-match was treated with selective IgG immunoabsorption (Imunosorba – Fresenius Medical Care), and became negative after the first procedure. The renal allograft recipients from ABO incompatible donor were pretreated with rituximab and plasmapheresis procedures [10–12]. The recipients with indexes near or over upper range in the mixed lymphocyte culture, beside standard triple immunosuppressive therapy received polyclonal (ATG-Fresenius – 3 mg/kg daily for 4 days) or monoclonal (daclisumab – 2 mg/kg, day 0 and 14) antibodies in the early postransplant period.

One patient received hyperimmune gamma globulin produced in our institution for the treatment of primary CVM infection [13].

The duration of follow-up in both groups was between 3 and 132 months, mean 41.1 ± 38.2 months.

We analyzed the differences between the groups in early surgical complications, episodes of acute rejection, cytomegalovirus infection, graft and patients survival and graft function, as assessed by serum creatinine level at the time of discharge after the transplantation and at the end of the follow-up period.

HLA system of donors and recipients was determined serologically. All patients had negative pretransplant lymphocytotoxic cross-match (complement dependent cytotoxicity – CDC). Titers of ABO isoagglutinine were measured using the standard procedure described previously [14]. Blood groups and Rh factor were tested with blood grouping reagents ABO-Rh / Reverse grouping cassette (Ortho-Clinical Diagnostics, USA). The third generation of ELISA was performed for detection of serological markers for viral hepatitis. Polymerase chain reaction (PCR) Amplicor (Hoffman la Roche) was used for viral RNA and DNA detection. Cut-off levels for cytomegalovirus was 400 copies/mL and for hepatitis C virus 2 000 copies/mL. Concentrations of serum creatinine were determined colorimetrically.

According to the protocol in our institution, the diagnosis of early acute rejection (in the first 15 postransplant days) was based on a combination of clinical presentation, laboratory findings and response to steroid pulse therapy. If there was no response to steroid therapy in three to five days the ultrasound-guided percutaneous graft biopsy was performed and tissue verification was obtained.

For data (frequencies) comparison between and within groups (inter- and intra-comparison) we used Kolmogorov-Smirnov test and ANOVA. The marked differences were significant at $p < 0.05$.

Results

The recipients in the groups were 8 males and 6 females, with the mean age of 49 ± 6.8 years in the group I and 47.5 ± 4.5 years in the control group (Table 1).

There were no significant differences between the groups according to sex, age, primary kidney disease, initial ESRF treatment, serology to hepatitis B virus (HBV) and hepatitis C virus (HCV), ABO compatibility and pretransplant cross match (Tables 1 and 2). As expected, a significant difference was in HLA matching, with worse matching of spousal grafts (Table 2). The spousal donors were not different significantly from the recipients in age, but related donors were significantly older ($p < 0.01$).

No significant difference was noticed between the groups in the duration of follow-up, surgical complications, episodes of acute rejection, cytomegalovirus (CMV) infection, graft outcome and patients survival. One patient from the group I had primary CMV infection as confirmed by CMV IgG D+/R-. Besides, six months of antiviral therapy

Table 1

Differences between the groups according to sex, age and primary kidney disease

Parameters	Groups		*p**
	Unrelated donor	Related donor	
Mean follow-up (months), $\bar{x}$ ± SD	41.14 ± 38.23	41.14 ± 38.23	ns
Total number of patients	14	14	
Sex			
male, n (%)	8 (57.1)	8 (57.1)	ns
female, n (%)	6 (42.9)	6 (42.9)	
Age (years), $\bar{x}$ ± SD	49.0 ± 6.82	47.5 ± 4.5	ns
Kidney disease, n (%)	1 (7.14)	3 (21.42)	ns

***Kolmogorov - Smirnov test**

Table 2

Differences between groups according to parameters in pretransplant period

Parameters	Groups		*p**
	Unrelated donor	Related donor	
First ESRD treatment, n (%)			
HD	12 (85.7)	11 (78.6)	
CAPD	0 (0.0)	1 (7.1)	ns
KTx	2 (14.3)	2 (14.3)	
Anti HCV+, n (%)	2 (14.3)	1 (7.1)	ns
HbsAg/HbeAg+, n (%)	1 (7.1)	0 (0.0)	ns
HLA matching; (years), $\bar{x}$ ± SD	39.23 ± 15.0	50.89 ± 3.34	0.01
ABO incompatibility, n (%)	2 (14.3)	1 (7.1)	ns

***Kolmogorov - Smirnov test**

ESRD – end stage renal failure; HD – hemodialysis; CAPD – continuous ambulatory peritoneal dialysis; KTx – kidney transplantation

with Ganciclovir (*iv* and oral), in early postransplant period he received hyperimmune gamma globulin produced in our institution.

Four patients from the group 1 had surgical complications but only one patient needed reoperation within the first 24 hours due to bleeding from the renal vein suture. One hemathoma and two seromas resolved spontaneously. Late phlebothrombosis of the right femoral vein in one patient was successfully treated with low molecular weight heparin.

Three patients from the control group had seroma, but only one needed surgery. One patient had hematoma which reabsorbed.

These surgical complications had no long-term consequences on graft function, as the serum creatinine returned to normal range after healing in all patients.

In an early postransplant period (first 7 days), six patients from the group I and three from the control group had episodes of acute rejection. These rejections were successfully treated with methylprednisolone pulses in the dose of 5–7 mg/kg. Only one patient from the group I (with ABO incompatible renal graft) had three repeated episodes of acute rejection in the first 10 months. During the postransplant period in this patient we failed to achieve the balance between immunosuppressive therapy and numerous opportunistic bacterial and viral infections, hypersensitive and myelotoxic effects of immunosuppressive, antiviral and antibiotic therapy. After 22 months she restarted hemodialysis and two months later died due to agranulocytosis.

One patient from the group I was HBs Ag and HBe Ag positive with normal liver function at the time of transplantation, and was not started on preventive antiviral therapy with lamivudine after transplantation. Four years later, HBV restarted replication, which could not be controlled with antiviral therapy (lamivudine), and eventually developed all the laboratory and clinical signs of liver cirrhosis. At the same time we noticed a gradual increase in serum creatinine levels, then increasing proteinuria to the nephritic range, followed by poorly controlled hypertension and finally progressive renal failure to ESRD. Due to serious coagulation problem graft biopsy was not done. Almost seven years after transplantation (82 months) he restarted hemodialysis.

With the exception of the two patients mentioned above, the remaining patients had stable renal allograft function during the follow-up. Neither group showed significant difference in graft function, evaluated by serum creatinine level, at the beginning and at the end of the follow-up. Estimated graft function also was not different between the groups, neither at the beginning nor at the end of the follow-up (Table 3).

Discussion

Transplantation is the best possible treatment for the end-stage kidney disease [1]. The gap between waiting list and demands for organs for a transplant program increases yearly [1]. Originally, kidney transplantation was performed only from well-matched living donors. Later, many states developed national programs for retrieving and distribution of organs from deceased donors [1–8]. With the advances in pretransplant evaluation, immunosuppressive protocols and postransplant management, short- and long-term outcomes have improved [1]. Kidney transplantation offered to transplanted patient almost normal life with only few discomforts: renal graft lifelong immunosuppressive therapy and moderate life style modifications.

Organ shortage was the reason for many countries to start considering transplantation from marginal deceased and living unrelated donors. Most of these countries have a transplant program from spousal donors, but some of them developed a program from other unrelated donors: distant relatives, paired-exchange, living deceased exchange, altruistic donations, not directed and directed donors [8].

In our institution, after an interview obtaining an informed consent and initial pretransplant evaluation, the Ethical Committee in each individual case gives a permission for the transplant program. Pretransplant evaluation is then completed and the couple is presented to the kidney transplant team, whose decision about medical indications for transplantation is final. Most of our spousal couples are deeply emotionally attached with their grown-up children. Their children show great interest in the transplant program for their parents with kidney disease. During the follow-up we noticed that the emotional and social connections between living donors and their recipients increased, and that they had positive influence on those who were in program for pretransplant evaluation and inspired the same couples to join the transplant program.

Similarly to the reports from other countries [2–8] our patients showed stable function during the whole follow-up period with the exception of two patients with significant pretransplant comorbidities.

The transplant program for spousal donors makes only 5% of total transplant program in our center. Similar percentage is found in other transplant centers worldwide. Some countries without a national program for transplantation from deceased donors formed an agency, which controls the program from living unrelated donors at the state level [5, 15]. Only one third of these donors are spouses, while the rest are volunteers, in the most cases young adults reimbursed for donation. Both the short- and the long-term outcome of such transplantations is better than from deceased donor.

Table 3

Differences in serum creatinine levels within and between the groups

Group	Creatinine at the start (μmol/L) $\bar{x} \pm SD$	Creatinine at the end (μmol/L) $\bar{x} \pm SD$	*p*
Related donor	120.93 ± 29.85	122 ± 41.2	0.73
Unrelated donor	124 ± 36.83	125.57 ± 17.54	0.86
p	0.81	0.77	

Special situation is found in the countries with illegal living unrelated kidney transplant program. The boards in many international transplant organizations are considering how to control such program, because they are aware of the fact that it is almost impossible to cease it.

Conclusion

As a transplant center we developed different modalities of living related donor programs: preemptively, during hemodialysis and CAPD, from ABO compatible and incompatible living related or unrelated-spousal donor. From 1996 to 2009 we performed only 14 transplantations from unrelated donors. Pretransplant evaluation of these couples was more demanding due to more detailed psychosocial evaluation and administrative procedure. In this limited number of cases the outcome and function of transplanted kidneys from unrelated donor did not differ from related donors during the follow-up period. The only source which could increase significantly kidney transplant program is organization of a national program for retrieval and distribution of organs from deceased donors.

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Implantacija savitljivih monoblok sočiva posle rupture zadnje kapsule tokom fakoemulzifikacije

Arteficial monoblock lenses implantation following rupture of posterior capsule during phacoemulsification surgery

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Apstrakt

Uvod/Cilj. Fakoemulzifikacija je savremena, suverena metoda operacije katarakte. Kroz minimalni rez (2,2–2,75 mm) na rožnjači vrši se emulzifikacija nukleusa sočiva pomoću ultrazvučnih talasa, a zatim i implantacija veštačkog sočiva u sočivnu vrećicu. Komplikacije tokom izvođenja fakoemulzifikacije su moguće kao i kod svake hirurgije i mogu varirati od bezazlenih pa do najtežih, sa posledičnim gubitkom vida. Jedna od mogućih komplikacija je i ruptura zadnje kapsule sočiva, koja može nastati u bilo kom stadijumu operacije. Cilj ove studije bio je da se ocene rezultati implantacije monoblok sočiva u sulkus sa osloncem na prednju kapsulu sa očuvanom kapsuloreksom posle rupture zadnje kapsule tokom fakoemulzifikacije. **Metode.** Sprovedena je prospektivna, nekomparativna studija u kojoj je praćeno 19 bolesnika kod kojih se, tokom operacije katarakte metodom fakoemulzifikacije, javila ruptura zadnje kapsule kao intraoperativna komplikacija. Prosečno vreme praćenja bilo je 14 (1–18) meseci. Posmatrana je najbolja korigovana vidna oštrina, intraokularni pritisak, nalaz na fundusu, kao i položaj implantiranog sočiva *wavelight allegro* okulajzerom (Scheimpflug kamera). **Rezultati.** Preoperativna vidna oštrina kretala se od osećaja svetla sa tačnom projekcijom (L+P+) do 0,5. Prvog postoperativnog dana zabeležena je vidna oštrina od 0,02 do 0,08 kod osam bolesnika, od 0,1 do 0,4, kod, takođe, osam bolesnika, od 0,5 i više kod tri bolesnik. Posle 12 meseci od operacije kod 15 bolesnika vidna oštrina bila je > 0,5. Od ranih postoperativnih komplikacija, edem rožnjače javio se kod šest bolesnika, pojačana reakcija u prednjoj komori sa ili bez fibrinske reakcije kod osam bolesnika i povećanje intraokularnog pritiska kod tri bolesnika. Sve promene bile su reverzibilnog karaktera. **Zaključak.** Ruptura zadnje kapsule jedna je od češćih i ozbiljnijih komplikacija fakoemulzifikacije koju je teško prevenirati. U zavisnosti od veličine rupture, moguće je izvršiti implantaciju monoblok savitljivih sočiva u sulkus sa osloncem na prednju kapsulu sa očuvanom kapsuloreksom kod najvećeg broja bolesnika, čime se zadržavaju prednosti malog reza: manji astigmatizam, bolja postoperativna vidna oštrina, ubrzano zarastanje rane, te ranija vidna i radna rehabilitacija.

Ključne reči:
hirurgija, oftalmološka, procedure; intraoperativne komplikacije; sočiva, intraokularna implantacija; fakoemulzifikacija; lečenje, ishod.

Abstract

Background/Aim. Phacoemulsification is a modern surgical technique for cataract operations. Through minimal corneal wound (2.2–2.7 mm) lens nucleus is emulsificated and arteficial lens is implanted in capsular bag. Complications during operations are possible, and can vary from minor to very serious one, with consecutive visual loss. One of possible complications is rupture of posterior lens capsule, which could happen in any stage of operation. The aim of this study was to evaluate results of monoblock arteficial lens implantation in sulcus on the remains of anterior capsule and capsulorhexis after posterior capsule rupture during phacoemulsification. **Methods.** This prospective, non-comparative study included 19 patients with rupture of posterior capsule as a result of cataract operation with phacoemulsification method. Average monitoring time was 14 months (1–18). We analysed best corrected visual acuity, intraocular pressure, fundus findings, and implanted lens position with wavelight allegro oculizer (Scheimpflug camera). **Results.** Preoperative visual acuity was from L+P+ to 0.5. On first postoperative day visual acuity 0.02–0.08 was noted in 8 patients, from 0.1–0.4 also in 8 patients and 0.5 and more in 3 patients. After 12 months from the operation 15 patients had visual acuity better than 0.5. Among early complications corneal edema was noted in 6 cases, anterior chamber reaction with or without fibrin reaction in 8 cases and rise of

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intraocular pressure in 3 cases. All complications were reversibile. **Conclusion.** Posterior capsule rupture/break is a serious complication of phacoemulsification, hardly to prevent. Regarding size of posterior rupture, foldable monoblock arteficial lens can be implanted into the sulcus on the remains of anterior capsule in most of the cases, keeping the advantages of small corneal incision: smaller astigmatism, better postoperative visual acuity, faster wound healing and earlier visual rehabilitation.

Key words:
ophtalmologic surgical procedures; intraoperative complications; lens implantation, intraocular; phacoemulsification; treatment outcome.

Uvod

Fakoemulzifikacija je savremena, suverena metoda operacije katarakte. Kroz minimalni rez (2,2–2,75 mm) na rožnjači vrši se emulzifikacija nukleusa sočiva pomoću ultrazvučnih talasa, a zatim i implantacija veštačkog sočiva u sočivnu vrećicu. Prednosti nad dosadašnjim metodama operacije katarakte su mnogobrojne, počevši od kraćeg postoperativnog oporavka bolesnika, manje izraženog astigmatizma, kraće vidne i radne rehabilitacije i većeg komfora za bolesnika i hirurga [1–6].

Komplikacije tokom izvođenja fakoemulzifikacije su moguće kao i kod svake hirurgije i mogu varirati od bezazlenih pa do najtežih, sa posledičnim gubitkom vida. Jedna od mogućih komplikacija je i ruptura zadnje kapsule sočiva, koja može nastati u bilo kom stadijumu operacije. Uprkos napretku hirurške tehnike i instumentarijuma, ovu komplikaciju je veoma teško prevenirati [7, 8]. U zavisnosti od veličine rupture zadnje kapsule i stadijuma operacije u kome se javila, kao i iskustva hirurga, odvija se i dalji tok operacije, vrši izbor intraokularnog sočiva za implantaciju i odluka o mestu implantacije – sočivna vrećica, sulkus, prednja komora, skleralna fiksacija i sl. [9].

Cilj ove studije bio je da se evaluiraju rezultati implantacije monoblok sočiva u sulkus sa osloncem na prednju kapsulu sa očuvanom kapsuloreksom posle rupture zadnje kapsule tokom fakoemulzifikacije.

Metode

U prospektivnoj, nekomparativnoj studiji pratili smo 19 bolesnika kod kojih se tokom operacije katarakte metodom fakoemulzifikacije javila ruptura zadnje kapsule kao intraoperativna komplikacija, i to u periodu od februara 2008. do februara 2009. godine. U tom periodu izvršeno je ukupno 758 operacija katarakte u Klinici za očne bolesti Vojnomedicinske akademije od strane istog hirurga. Kod 19 bolesnika došlo je do rupture zadnje kapsule tokom operacije, ali je bilo moguće implantirati monoblok savitljivo sočivo u sulkus sa osloncem na prednju kapsulu sa očuvanom kapsuloreksom bez širenja kornealnog reza. Od tog broja, šest bolesnika bilo je muškog, a 13 ženskog pola. Bolesnici su praćeni prosečno 14 meseci (raspon 1–18 meseci).

Operacije su izvođene u topikalnoj anesteziji, metodom fakoemulzifikacije sa trostepenim kornealnim rezom i sa dva paracentezna otvora. U situaciji kada bi došlo do komplikacije – rupture zadnje kapsule, dalji tok operacije i pristup hirurga zavisio je od stadijuma u kome je došlo do komplikacije. Korišćeni su različiti pristupi da bi se sprečila ili zaustavila dalja pojava staklastog tela, dalje širenje rupture i da bi se održao stabilni zatvoreni sistem u daljem toku operacije. Ukoliko je bilo potrebno, vršena je i prednja vitrektomija. Sočivo je implantirano u sulkus sa osloncem na prednju kapsulu sa očuvanom kapsuloreksom u svim slučajevima kada je procenjeno da postoji adekvatna podrška prednje ili ostataka zadnje kapsule prirodnog sočiva. Postoperativno, primenjivana je standardna terapija posle operacije katarakte. Ukoliko je bilo potrebno, uključivana je i antiglaukomna (topikalna i/ili oralna) ili dodatna antiinflamatorna terapija (diklofenak). Bolesnici su praćeni 1, 3, 7 i 30 postoperativnih dana, a zatim i 3, 6, 12 i 18 meseci, prosečno 14 meseci. Posmatrana je najbolja korigovana vidna oštrina, intraokularni pritisak, nalaz na fundusu, kao i položaj implantiranog sočiva *wavelight allegro* okulajzerom (Scheimpflug kamera).

Rezultati

Ruptura zadnje kapsule javljala se u različitim stadijumima operacije. Izvršeno je i devet prednjih vitrektomija, ali nije bilo potonuća nukleusa ili drugih fragmenata sočiva u staklasto telo (tabela 1).

Tabela 1
Stadijum operacije i vreme pojave rupture zadnje kapsule

Stadijum operacije	Broj očiju n (%)	Izvršena prednja vitrektomija n (%)
Fakoemulzifikacija	12 (63)	7 (58)
Irigacija/ aspiracija	4 (21)	1 (25)
Implantacija IOL	2 (11)	1 (50)
Poliranje zadnje kapsule	1 (5)	0 (0)
Ukupno	19 (100)	9 (48)

IOL – Intraokularno sočivo

Preoperativna vidna oštrina kretala se od osećaja svetla sa tačnom projekcijom (L+P+) do 0,5. Prvi postoperativni dan zabeležena je vidna oštrina od 0,02–0,08 kod osam bolesnika, od 0,1–0,4 kod osam bolesnika, od 0,5 i više kod tri bolesnika. Od ranih postoperativnih komplikacija, edem rožnjače javio se kod šest bolesnika, pojačana reakcija u prednjoj komori sa ili bez fibrinske reakcije kod osam bolesnika, prolazno povećanje intraokularnog pritiska kod tri bolesnika (tabela 2).

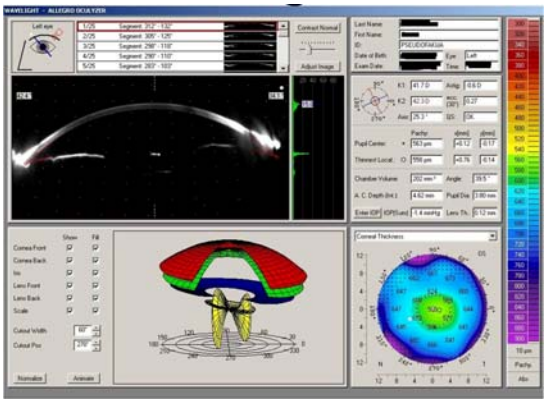
Tabela 2
Najčešće postoperativne komplikacije naših bolesnika

Komplikacija	Broj očiju
Edem rožnjače	6
Pojačana inflamatorna reakcija u prednjoj komori	8
Skok intraokularnog pritiska	3
Nepravilnost zenice	1
Rezidualni korteks	1

Manji rezidualni nabubreli korteks bio je prisutan kod jednog bolesnika, kao i lako nepravilna zenica (zaostala traka staklastog tela). I prva i druga komplikacija uočene su tek nekoliko dana posle operacije (posle bubrenja delova pretežno providnog korteksa sočiva), te perifernog manjeg prolapsa vitreusa sa blagom decentracijom zeničnog otvora. Edem rožnjače bio je i razlog za slabu vidnu oštrinu prvog postoperativnog dana, ali se na primenjenu standardnu postoperativnu terapiju povukao za 7–10 dana u svim slučajevima.

Povišen intraokularni pritisak lečen je topikalnim antiglaukomnim lekovima i bio je prolaznog karaktera kod dva bolesnika, dok je jedan bolesnik nastavio sa antiglaukomnom terapijom koju je imao uvedenu i ranije (simpleks oblik glaukoma). Pojačana inflamatorna reakcija zahtevala je dodatnu terapiju kod dva bolesnika. Manji rezidualni korteks resorbovao se pet nedelja posle operacije i nije zahtevao dodatnu hiruršku intervenciju. Na kontroli posle mesec dana, vidna oštrina 0,02–0,08 zabeležena je kod četiri bolesnika, od 0,1 do 0,4 kod četiri bolesnika, i 0,5 i više kod 11 bolesnika (tabela 3).

rifikovan je i profilnim snimkom prednjeg segmenta *wavelight allegro* okulajzerom. Uočena je decentracija intraokularnog sočiva (IOL) manjeg stepena kod dva bolesnika koja nije zahtevala dodatnu intervenciju (slika 3).



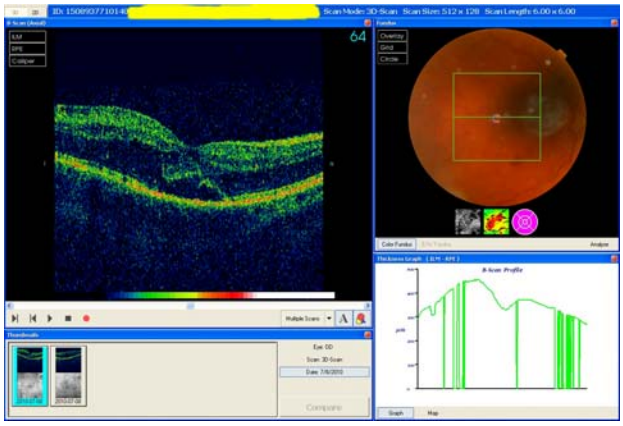
Sl. 3 – Prikaz decentriranog intraokularnog sočiva (IOL) četiri meseca posle operacije

Tabela 3

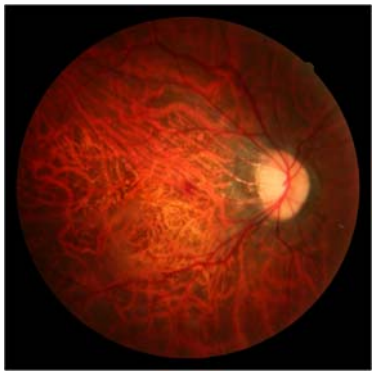
Postoperativna vidna oštrina

Vidna oštrina	Posle mesec dana	Posle šest meseci	Posle 12 meseci	Posle18 meseci
0,02–0,08	4	2	2	2
0,1–0,4	4	3	2	2
> 0,5	11	14	15	15

Uzrok slabe vidne oštrine kod dva bolesnika bila je pojava cistoidnog edema makule, kod jednog, senilna degeneracija žute mrlje (slika 1) i miopna makulopatija, takođe, kod jednog bolesnika (slika 2). Položaj implantiranog sočiva ve-



Sl. 1 – Senilna degeneracija žute mrlje



Sl. 2 – Miopne degenerativne promene

Diskusija

Do komplikacija u hirurgiji katarakte metodom fakoemulzifikacije može doći u praktično svakoj od faza operacije. Ruptura zadnje kapsule je jedna od češćih i ozbiljnijih komplikacija koju je teško prevenirati. Može se javiti u svim stadijumima operacije, ali je po našim iskustvima najčešća tokom faze emulzifikacije nukleusa i faze irigacije/aspiracije, što je u skladu sa podacima iz skorijih radova [2–6].

U zavisnosti od veličine rupture, moguće je izvršiti implantaciju monoblok savitljivih sočiva kod najvećeg broja bolesnika, u sulkus sa osloncem na prednju kapsulu sa očuvanom kapsuloreksom. Ukoliko je moguće, treba izbegavati širenje reza za implantaciju sočiva, jer se na taj način gube prednosti malog reza – manji astigmatizam, ubrzano zarastanje rane, rana vidna i radna rehabilitacija [3, 7–11].

U našoj seriji od 19 bolesnika, izvršili smo implantaciju monoblok savitljivih sočiva kroz postojeću inciziju ili je izvršeno širenje reza na 3,2 mm. Sočiva su ugrađena u sulkus sa osloncem na prednju kapsulu sa očuvanom kapsuloreksom. Kod devet bolesnika izvršena je i prednja vitrektomija.

Prema podacima iz literature, najčešća komplikacija bila je edem rožnjače i pojačana inflamatorna reakcija u prednjoj komori [12–14].

U našoj seriji, najčešće rane postoperativne komplikacije takođe su bile edem rožnjače i pojačana inflamatorna reakcija u prednjoj komori. Edem rožnjače na primenjenu terapiju povlačio se za 7–10 dana, bez pojave bulozne keratopatije uključujući i bolesnika je vršena prednja vitrektomija.

Inflamatorna reakcija u prednjoj komori sa ili bez fibrina očekivana je kao posledica rupture zadnje kapsule. Na primenjenu antiinflamatornu terapiju i ona se povlačila 3–5

dana posle operacije, iako je kod dva bolesnika uvedena i dodatna antiinflamatorna terapija (diklofenak kapi).

Povišenje intraokularnog pritiska kod dva bolesnika bilo je prolazne prirode i na primenjenu topikalnu antiglaukomnu terapiju pritisak se stabilizovao posle 2–3 dana. Jedan bolesnik nastavio je sa antiglaukomnom terapijom koju je imao i pre operacije katarakte. Kraći prolazni skok intraokularnog pritiska može se objasniti zaostacima viskoelastika u prednjoj komori [9, 14].

Zadovoljavajuća postoperativna vidna oštrina posle šest meseci postignuta je kod 17 bolesnika (tabela 3). Kod preostala dva bolesnika, senilna degeneracija žute mrlje i miopna makulopatija bili su odgovorni za slabu vidnu oštrinu (slike 2 i 3).

Položaj implantiranog sočiva verifikovan je i profilnim snimkom prednjeg segmenta *wavelight allegro* okulajzerom, koji je pokazao decentraciju IOL manjeg stepena kod dva bolesnika. Upotreba okulajzera koji poseduje Scheimpflug kameru je nezvanični zlatni standard za procenu položaja IOL [15, 16].

Manja deformacija zeničnog otvora usled trake vitreusa, takođe, nije zahtevala dodatnu hiruršku intervenciju, već je po njenom fibroziranju (tri meseca posle operacije) izvršeno njeno presecanje Neodimium Yag laserom.

Zaključak

Ruptura zadnje kapsule tokom fakoemulzifikacije jedna je od češćih i ozbiljnijih komplikacija koju je teško prevenirati. U zavisnosti od veličine rupture, moguće je izvršiti implantaciju monoblok savitljivih sočiva u sulkus sa osloncem na prednju kapsulu sa očuvanom kapsuloreksom kod najvećeg broja bolesnika, čime se zadržavaju prednosti malog reza: manji astigmatizam, bolja postoperativna vidna oštrina, ubrzano zarastanje rane, te ranija vidna i radna rehabilitacija.

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Recent views on cytohistological characteristics and pathogenic mechanisms of atherosclerotic lesions types I, II and III

Savremeno shvatanje citoloških karakteristika i patogenih mehanizama aterosklerotskih bolesti tipa I, II i III

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Key words:
arteriosclerosis; infection; inflammation; inflammation mediators; histocytochemistry; pathology, clinical.

Ključne reči:
arterioskleroza; infekcija; zapaljenje; zapaljenje, medijatori; histocitohemija; patologija, klinička.

Introduction

Contemporary model of the pathogenesis of atherosclerosis is based on the hypothesis that local damage of the endothelium together with systemic factors such as hypercholesterolemia, hyperglycemia, hypertension, chronically infections, genetic factors, *diabetes mellitus*, initiate a cascade of processes which eventually cause atherosclerotic lesion development [1, 2].

Although various hypotheses are already known about the pathogenesis of atherosclerosis and different factors of predisposition have been studied, from the point of molecular and vascular biology to clinical presentations and therapy methods, in modern literature there are still opposing views about the initial moment in the pathogenesis of this condition, regarding its functional and morphological changes.

The stage of the initial lesion - early lesion (type I)

Activation of endothelium

The initial stage, which precedes the development of atherosclerosis, is endothelial dysfunction, namely, activation of endothelial cells as a specific response to the action of harmful agents. Acute response of endothelium results in inflammation, coagulation disorders and vasomotor changes. The release of inflammation mediators deposited in the Weibel Palade bodies represents a very quick response of endothelial cells [3]. One of the most important consequences of endothelial activation is a decreased NO production, which causes the absence of its antiatherogenic, antiproliferative and vasodilatatory effects and, also, causes an increased production of endothelin-1 (ET-1) which in turn, through its mitogenic effect, stimulates proliferation of smooth muscle cells (SMCs) in the intima, and consequently initiate atherosclerosis [4, 5].

During activation of endothelial cells, changes in the process of coagulation have also been observed. Endothelial cells modulate their phenotype from anticoagulative to procoagulative, through an increased expression of tissue factors, or increased release of tissue plasminogen [6, 7].

Besides alterations in tonus and coagulation, endothelial activation includes increased expression of adhesive proteins (P-selectin, integrins) which promote the adhesion of leukocytes on endothelium and their infiltration in the subendothelial connective tissue [8]. This results in the release of free radicals, proteases and elastases which lead to further damage of endothelial cells [7].

Accumulation of lipids in the intima

The first step in the development of atherosclerosis is the accumulation of lipid droplets in the intima of the vessel

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wall, as a consequence of hypercholesterolemia, coupled with systemic hypertension. Lipid droplets bind to proteoglycans in the intimal connective tissue and have a tendency to form aggregates [9]. Lipoproteins in the lipid droplets may oxidize or become chemically modified in other ways [10, 11].

Hypothesis of oxidative modification

Oxidative modification is the best researched study about the modification of lipoproteins (LDL) in atherosclerosis. Studies showed that oxidized LDL (ox-LDL) has been intensely absorbed in the culture of macrophages, partially through SR-A receptor, but in a larger part through other scavenger receptors, especially CD36. Circulating monocytes express SR-A and CD36, but at a lower degree. However, when monocyte enters into vascular wall and transforms to macrophage, during this transformation the expression of scavenger receptors is increased, and this allows absorption of modified LDL forms [12].

The rate of production of ox-LDL in the arterial intima is related to the concentration of native LDL. Concentration of native LDL in the intima depends on its concentration in plasma. These results have shown that possible predilected places for pathogenesis of atherosclerotic lesions could be thickenings of the intima on the proximal parts of the artery, after their branching or bifurcation [13].

Furthermore, it has been experimentally approved that the concentration of LDL in the vascular intima is higher in places where atherosclerosis will develop, compared to the places which are resistant [14]. It is interesting that permeability of the overlying endothelium is not increased, which means that LDL somehow selectively accumulates in those places. It is proven that LDL in these predilection places binds to proteoglycans of extracellular matrix [9]. Lipid droplets binded to proteoglycans in this way oxidize much more easily [15–17]. Also according to the literature, genetically engineered LDL does not bind to proteoglycans, which means it is less pathogenic than native LDL [18]. The presence of SMCs of synthetic phenotype contributes to the accumulation of LDL in intima. The number of these cells is increased in initial lesions; they act as fibroblasts and synthesize large amounts of proteoglycans. Previously presented facts suggest that the accumulation of LDL in intima could be the initial step in the pathogenesis of atherosclerotic lesion [16, 17].

At the initial lesions beside the increased expression of the vascular cell adhesion molecule-1 (VCAM-1), which selectively promotes the adhesion of lymphocytes and monocytes, ox-LDL itself contributes to the adhesion of these cells on the endothelial layer [18]. Oxidized LDL has cytotoxic effect on endothelial cells, acts as a mitogen of macrophages and SMCs and stimulates the release of monocyte chemoattractant protein-1 (MCP-1) and monocyte colony-stimulated factor (M-CSF) from endothelial cells, which directly starts chemotaxis and migration of monocytes into the subendothelial layer [19].

Degradation of polyunsaturated fatty acids in sn-2 place of phospholipids in LDL generates ox-LDL. After generation, ox-LDL binds to proteoglycans and becomes uptaken by macrophages through scavenger receptors [20].

Expression of adhesion molecules in atherosclerosis

After initiation by hypercholesterolemia, leukocytes (at first, monocytes and T lymphocytes) adhere to the endothelium and actively migrate both, between and through endothelial cells into the intima [21]. In the subendothelium of the intima monocytes begin to accumulate lipids and begin transforming into foam cells. In that manner, increased expression of adhesion molecules, as a consequence of endothelial dysfunction and the accumulation of lipid droplets in the intima, promote adhesion of leukocytes to the endothelial layer and their migration into the subendothelium, in the initial and advanced stages of atherosclerosis [22–24].

A variety of adhesion molecules have been identified as important for this process. These include immunoglobulin superfamily members intercellular adhesion molecules 1 and 2 (ICAM-1 and ICAM-2), vascular cell adhesion molecule 1 (VCAM-1), platelet-endothelial cell adhesion molecule-1 (PECAM-1), as well as E-, P- and L-selectin [21, 25, 26].

Increased expression of VCAM-1 is a characteristic of an initial stage of atherosclerosis. This molecule interacts with specific integrin, very late antigen-4 (VLA-4), which is expressed only on monocytes and lymphocytes [25, 27]. As a result of this fact, at the initial stage of atherosclerosis, monocytes and T cells adhere in a significantly higher percentage than other types of leukocytes [28, 29]. On the other hand, ICAM-1 allows adhesion of a number of different leukocytes, because it interacts with CD11a (LFA-1) and CD11b (Mac-1) integrins on their membranes, and its expression is a characteristic of advanced atherosclerotic lesions [25, 27].

E-selectin (previously ELAM-1) is a typical representative of this group of adhesion molecules. Its name derives from a specific expression only on endothelial cells. Its increased expression is a characteristic of the advanced atherosclerotic lesions. This molecule interacts with Sialyl-Lewis X ligand on the membrane of granulocytes and memory T cells and due to this fact, at the advanced atherosclerotic lesions, these cells adhere to endothelial layer in a significantly higher percentage [25, 27].

P-selectin (previously GMP-140) is expressed primarily in thrombocytes, but also in overlying endothelium above atherosclerotic lesions. This molecule interacts with P–selectin glycoprotein ligand-1 (PSGL-1) on monocytes, lymphocytes and granulocytes. It has a significant role in „rolling“ of leukocytes on endothelial layer above the lesion [30–32].

L-selectin is expressed on leukocytes and interacts with PSGL-1 ligand on monocytes, lymphocytes and granulocytes, but also with mucosal vascular addressin cell adhesion molecular link-1 (MAdCAM-1) [25, 27, 31].

In the endothelium above an atherosclerotic plaque, in the sites of infiltration of macrophages, there is a noticeable increase of expression of ICAM-1 and P-selectin [21, 23, 30]. These results indicate that the accumulation of monocytes which will later transform into macrophages partially dependes on synergic action of P-selectin and ICAM-1 on the endothelial layer [31, 32].

Many factors combined can induce an expression of endothelial adhesion molecules in atherosclerosis [31]. These

factors are ox-LDL, inflammatory cytokines and biomechanical strength of blood shear stress. In the tissue culture, it is noticeable that the treatment of endothelial cells with ox-LDL increases the expression of P-selectin, ICAM-1 and VCAM-1 [32, 33]. Cytokines, including interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), increase the infiltration of leukocytes through adhesion molecules [2]. Regarding biomechanical stimuli, it is shown that the regulation of ICAM-1 expression is under influence of blood flow [34].

Migration of leukocytes through endothelial layer

When leukocytes adhere to endothelium, they must receive a signal in order to go through endothelium into the deeper layers of vascular wall. Two characteristic processes, selectin-mediated rolling and integrin-mediated adhesion, cooperate to promote active migration of leukocytes, both across endothelial cells (transendothelial migration) and by diapedesis. Rolling of leukocytes and migration into the vascular wall are the crucial first steps in the development of inflammation and atherosclerosis [21].

Contemporary concepts regarding direct migration of leukocytes include activation of special cytokines-chemokines. At the early stage of atherosclerosis, one of those cytokines, MCP-1 is being produced in the endothelial cells, as a response to ox-LDL and other stimuli [34, 35]. This cytokine directly starts chemotaxis and the migration of monocytes into the subendothelial layer. At this stage, subpopulations of intimal SMCs and endothelial cells also express M-CSF which also promotes chemotaxis of monocytes, their adhesion and differentiation to macrophages, regulate proliferation of macrophages and other types of cells, which is implicated in inflammatory-fibroproliferative response at the advanced stages of atherosclerosis. Oxidized LDL also stimulates endothelial cells to express granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF). These growth factors affect differentiation, survival, proliferation, migration and metabolism of macrophages/granulocytes, and G–CSF and GM–CSF also affect migration and proliferation of endothelial cells [32–35].

Other types of chemokines may also increase lymphocyte accumulation in plaque. In atherosclerotic lesion, overlying endothelium expresses three types of lymphocyte-selective chemokines: interferon-inducible protein-10 (IP-10), interferon-inducible T-cell alpha chemoattractant (I-TAC) and monokine induced by interferon-γ (MIG). Interferon-γ which is present in the atherosclerotic lesion induces genes which code T cell hemoattractant protein family of chemokine, and subsequently, accumulation of T cells in plaque [36, 37].

Foam cells of monocyte-macrophage lineage

When monocytes enter the intima of a vessel wall, they undergo phenotype modification to macrophages. Macrophages then accumulate lipid droplets and become foam cells. The mechanism of cholesterol uptaking in macrophages is especially interesting.

Most of the cells use a plasma membrane receptor to uptake and release cholesterol. When a cell uptakes cholesterol for its own metabolic needs, a mechanism of the transcription control of this receptor become activated, which in turn shuts down the receptor expression and completely shuts down further absorption of cholesterol. On the contrary, macrophages in the atherosclerotic lesion (future foam cells) do not posses this kind of negative feedback, so it is obvious that they uptake cholesterol through some other kind of receptors [38].

Some investigations have shown that in the culture of macrophages in *in vitro* conditions, cells rapidly uptake modified, acetylated LDL, not through the known LDL receptor, but through the special "acetyl-LDL" receptor. Later, this receptor is cloned and called the scavenger receptor A (SR-A). Contrary to LDL receptor, expression of SR-A is not down-regulated as a response to the increased intracellular concentration of cholesterol. Theoretically, this kind of receptor might have a role in forming foam cells, because there is no data that acetyl-LDL has been generated in *in vivo* conditions [39, 40]. According to literature, the various modified forms of LDL also could have a role in the creation of foam cells, which brings out the question of the existence of various receptors [41, 42].

Other studies [42–44] have shown that some foam cells originate from SMCs, probably due to the fact that SMCs can also express scavenger receptors if they are properly activated, which will be discussed later.

Cytohistological characteristics of an initial lesion – early lesion (type I)

The results of a large number of recent studies on cytohistological characteristics of atherosclerotic lesions have shown that at the stage of initial lesion (early lesion – type I) there are no visible morphological changes in the structure of vascular wall, which is in accordance with the previously stated pathogenic mechanisms. According to these results, at the initial stage endothelial continuity is preserved, with the presence of individual foam cells and T cells in the intima. At initial phases of lesions circulating monocytes are the main precursors to foam cells. Smooth muscle cells in the intima and media show contractile phenotype (Figure 1). Their phenotype modulation from the contractile to synthetic phenotype can be noticed during the fatty streak stage [28, 45, 46].

The fatty streak stage - early lesion (type II)

Cytohistological characteristics of the fatty streak stage – early lesion (type II)

With further development of atherosclerotic lesion, at the fatty streak stage (early lesion – type II) increase in the number of foam cells (Figure 2) and intense infiltration of T cells occurs. At this stage, lipid accumulation in foam cells forms fatty streaks. Endothelial layer is still morphologically preserved, although it is functionally damaged [46, 47].

Foam cells at this stage originate from macrophages (Figure 3) [46, 47]. The fatty streak stage is also characterized by the presence of SMCs of synthetic phenotype, but they are not foam cells yet [29, 47].

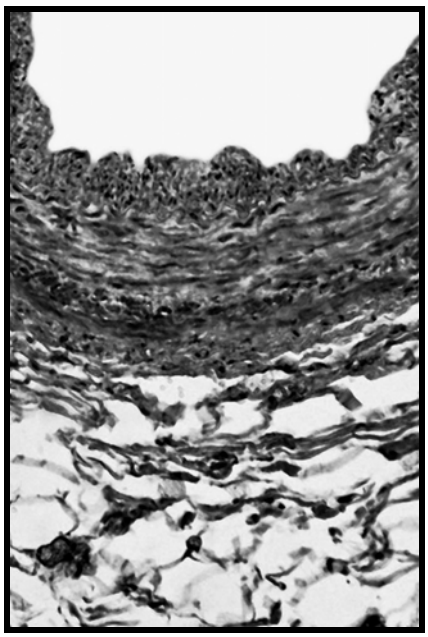

Fig. 1 – Coronary atherosclerosis at the initial stage. Endothelial layer is well-preserved without visible morphological changes. Smooth muscle cells in intima and media show contractyle phenotype (immunohistochemical staining of MHC, original magnification ×64)

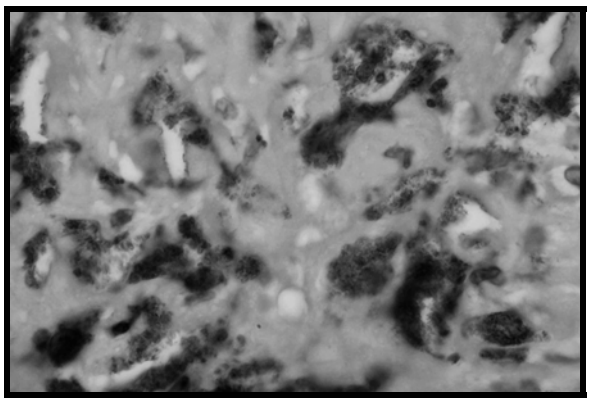

Fig. 2 – Coronary atherosclerosis at the fatty streak stage. Foam cells in subendothelial layer (immunohistochemical staining of CD68, original magnification ×256)

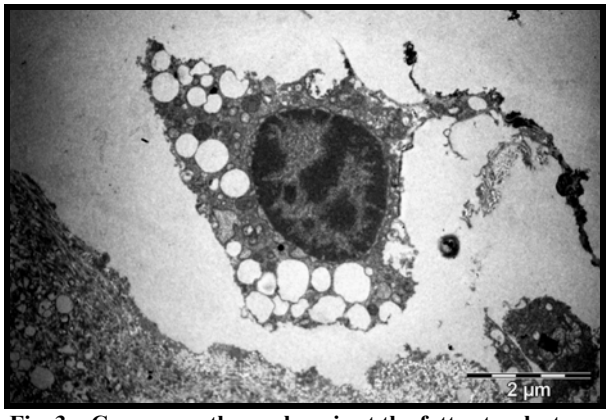

Fig. 3 – Coronary atherosclerosis at the fatty streak stage. Macrophage with a large number of lipid inclusions in cytoplasm, *ie* foam cell that originate from the monocyte-macrophage lineage (TEM, original magnification ×16875)

Smooth muscle cells (SMCs)

The results of testing phenotypical status of SMCs of atherosclerotic lesions showed that from a fatty streak stage, the modified SMCs of synthetic phenotype form the dominant cell population [28]. The development of synthetic phenotype is followed by a reduction in myofilaments [29]. Smooth muscle cells of synthetic phenotype expressed α-smooth muscle actin (α-SMA) and vimentin, with a lack of expression of desmin [43, 48]. The loss of desmin expression with concurrent vimentin expression is the first sign in the process of switching from contractile to synthetic phenotype [43, 49]. According to the existing literature, vimentin is an intermediary filament that can be found in differentiated SMCs as well, but it is coexpressed there with desmin. With the loss of contractile phenotype and characteristic desmin expression (*i.e.* with switching of cells to synthetic phenotype), the expression of vimentin filaments can be noticed [28, 45, 50].

Switching of SMCs to synthetic phenotype is a main characteristic of vascular remodeling during the pathogenesis of atherosclerosis [48, 51]. Vascular remodeling is an adaptive process involving the adjustment of structure and function of blood vessels to long-term changes in hemodynamic conditions, especially to hypertension [45, 52]. Scientific reports prove that reactive – adaptive intimal proliferation ("early response" of the wall) during the process of vascular remodeling, does not have a mechanism of negative feedback and that it can be continued in the form of intimal hyperplasia. In the primary response to the conditions of increased pressure, there is a proliferation of endothelial cells in vascular wall. After endothelial proliferation, during a rather long period of time it is characterized by SMCs proliferation accompanied by vascular wall thickening. The increased synthesis of collagen and elastin, which is an alteration of gene expression for the synthesis of these proteins, is the explanation for rapid enlargement and irreversible thickening in atherosclerotic vascular remodeling, in spite of apoptosis which is activated after proliferation of SMCs [52, 53].

Under the influence of chemotactic factors, SMCs migrate through fenestrae of the internal elastic lamina. As they express specific integrins (α2β1 which binds collagen and α3β1 and α5β1 which bind fibronectin) and elastin binding proteins on plasma membrane, they come in contact with connective tissue components and start to proliferate as a response to the stimuli of platelet-derived growth factor (PDGF) and fibroblast growth factors (FGFs) [55]. At the same time, under the influence of TGF-β, SMCs begin to secrete extracellular matrix components, thereby increasing the amount of collagen IV and collagen V. These changes were associated with up regulation of transforming growth factor β (TGF- β) [52].

Also, at this stage, adventitial myofibroblasts start to show the characteristics of contractile SMCs [29, 47, 48]. According to the same data, the increased expression of TGF-β in adventitia is the signal for differentiation of fibroblasts (which start to express α-SMA and the other markers of SMCs differentiation as well) and their migration, which makes it hard to differentiate from medial SMCs (Figure 4).

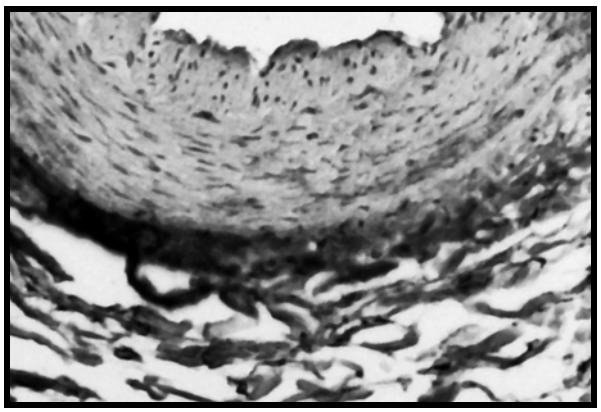

Fig. 4 – Coronary atherosclerosis at the fatty streak stage. Myofibroblasts in adventitia start to show the characteristics of contractile smooth muscle cells (immunohistochemical staining of MHC, original magnification ×64)

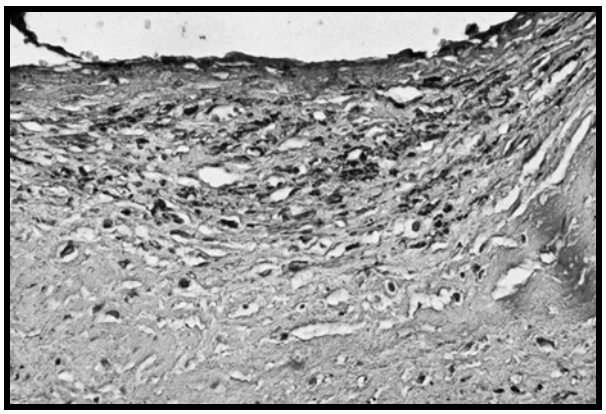

Fig. 5 – Coronary atherosclerosis at the preatheroma stage. Smooth muscle cells of the synthetic phenotype (immunohistochemical staining of vimentin, original magnification ×128)

A number of important stimulators and inhibitors of both SMCs and endothelial cells in atherosclerotic lesions have now been purified and biochemically characterized. Included in the group of stimulators are FGFs, vascular endothelial growth factor (VEGF), PDGF, epidermal growth factor (EGF) like factors, angiogenin, also vasoconstrictor peptides (which induce proliferation of SMCs and the expression of TGF-β and PDGF), angiotensin converting enzyme (ACE) (which induces proliferation of SMCs in *in vivo* conditions), insulin-like growth factors (IGFs) (potential mitogen of SMCs), interleukin-1 (IL1) (induces expression of FGF in SMCs), lipoprotein A (stimulates proliferation of SMCs) and ET-1 [22, 54].

Bifunctional growth factors, TGF-β and TNF-α, also have an influence on the proliferation and migration of SMCs. A multifunctional cytokine is TGF-β, which in its inactive form is being synthesized by endothelial cells and SMCs. This cytokine becomes activated under the influence of plasmin and induces differentiation of endothelial cells and SMCs, but paradoxically inhibits their migration and proliferation. TNF-α is cytokine which inhibits proliferation of endothelial cells and SMCs but it stimulates the transcription of heparin-binding EGF-like growth factor (HB-EGF) in SMCs [31, 54]. Each of these factors may influence the growth of SMCs or endothelial cell, both as a stimulant or inhibitor.

The preatheroma stage – intermediary lesion (type III)

Cytohistological characteristics of the preatheroma stage – intermediary lesion (typeIII)

At the preatheroma stage – intermediary lesion (type III), lipid accumulation surpasses the accumulation by foam cells and subendothelial layer contain scattered collections of extracellular lipid droplets and particles in the form of small isolated pools of extracellular lipid. The preatheroma stage is characterized by the presence of intimal and medial proliferating SMCs of synthetic phenotype (Figure 5). The phenotype modulation of intimal and medial SMCs (based on the loss of desmin expression and the appearance of vimentin expression) begins at the fatty streak stage, and at the preatheroma stage these cells form the dominant cell population [28, 29, 45].

The intermediary lesion is also characterized by a large number of foam cells of various origin. Some of foam cells develop from monocyte-macrophage lineage (the same as at the fatty streak stage) and others originate from SMCs [28, 46, 47]. Foam cells that originate from SMCs are spindle-shaped or star-shaped and they have short, thick extensions with lipid inclusions in cytoplasm (Figure 6) [28, 48].

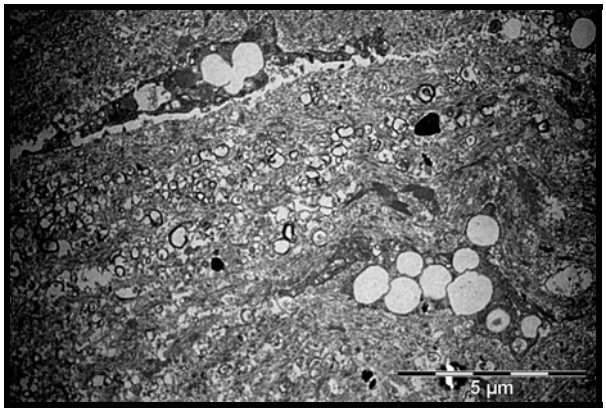

Fig. 6 – Coronary atherosclerosis at the preatheroma stage. We can notice the presence of foam cells that originate from smooth muscle cells. These lipid-laden smooth muscle cells are spindle-shaped or star-shaped, with lipid inclusions in the cytoplasm and they have short, thick extensions. The number of lipid inclusions in the cells varies, and therefore, they look as if they are at the different stages of phenotype transformations to foam cells (TEM, original magnification ×8250)

Foam cells of smooth muscle cells origin

The results of many studies have shown that SMCs of synthetic phenotype, proliferate and start to accumulate lipids from the fatty streak stage, but their largest number can be observed at the preatheroma stage [28, 29, 45]. According to the same data, some foam cells in atherosclerosis originate from SMCs of synthetic phenotype (express vimentin-immunoreactivity), probably due to the fact that some SMCs can also express scavenger receptors. Vimentin-immuno-

reactivity points out to their syntetic/proliferative activity and also to their mesenchymal origin [45, 46].

The fact that SMCs express scavenger receptors and modulate to foam cells is possibly related to the embryonic origin of vascular SMCs [44]. All vascular SMCs have a mesenchymal origin, except for the fact that large arteries in the upper parts of the body can have the neuroectodermal origin [28, 56, 57]. Due to their mesenchymal origin, SMCs coexpress vimentin and desmin. During remodeling of vascular wall in atherosclerosis, there is a loss of contractile characteristics of SMCs and characteristic desmin expression so that SMCs of the synthetic phenotype express only vimentin [28, 29, 43]. After loss of contractile characteristics, SMCs proliferate and as a result of this proliferation start to intensively express S-100 protein. For both, SMCs of mesenchymal origin, as well as for SMCs of neuroectodermal origin, expression of this protein is a normal characteristic, but it is increased in processes which are Ca^{++} – mediated. This protein in increased expression shows a high affinity to bind unsaturated lipid acids [58]. It has been assumed that SMCs due to their synthetic and proliferative activity can accumulate lipids.

Pathogenesis of atherosclerosis as an inflammatory response of vascular wall

Earlier theories about the pathogenesis of atherosclerosis have assumed hypercholesterolemia as a necessary condition for the development of this disease. Only in the later decades, numerous researches have pointed out to inflammation as a basis for pathogenesis of atherosclerosis [27].

As mentioned above, beginning with the initial lesion stage, in the response to the presence of modified lipids in the subendothelial layer, endothelial cells increase the expression of adhesion molecules, at first VCAM-1, which selectively promotes the adhesion of monocytes and T cells on endothelial layer. In response to the same stimulus endothelial cells express monocyte-selective chemokine MCP-1 and three types of lymphocyte-selective chemokines (IP-10, I-TAC and MIG) which directly start chemotaxis and migration of monocytes and T cells into the subendothelial layer. The presence of macrophages and T cells in subendothelial layer show that the early stages of atherosclerotic lesion are an inflammatory response to exogenous pathogens [27, 51].

However, in addition to modified lipids, other exogenous pathogens, especially microorganisms, also promote inflammation. These antigens become presented by antigen-presenting cells (APCs), to Th CD4+ cells trough the MHC class II-dependent pathway. In response to immunological activation, mature forms of Th CD4+ cells begin to produce proinflammatory cytokines IL-2, IFN-γ and TNF-β. These cells also interreact with Tc CD8+ cells, NKT cells and macrophages, helping them to finish a started immune response against intracellural pathogens [27].

The role of APCs during atherosclerosis is played by macrophages, vascular dendritic cells (VDCs) and B cells, as a "professional APCs", but recent studies have show that low differentiated forms of SMCs also can process and present antigens [59, 60]. All APCs in vascular wall internalize antigens either by phagocytosis or by receptor-mediated endocytosis, and display a fragment of antigen bound to the class II MHC molecule on their plasma membrane. Expression of the antigen-class II MHC molecule complex which is a ligand for a T-cell receptor (TCR), on the membranes of APCs with additional co-stimulatory signals from APCs leads to immunological activation of T cells and manifestation of inflammatory reactions [27, 59, 60].

A great number of recent studies aimed to improve therapeutic procedures have been focused on the role of IFN-γ during atherosclerosis. Immediately after entrance into a subendothelial layer, T cells produce IFN-γ which induces genes that code T cell hemoattractant protein family of chemokine, and subsequently, further accumulation of T cells in the plaque. Besides, this cytokine activates macrophages immunoregulatory functions and simultaneously affects SMCs of a lesion. These literature data support the hypothesis that IFN-γ is a potent atherogenic cytokine, which significantly increases both, lesion size as well as the number of T lymphocytes within lesions, and also up regulates PDGF which causes an increased migration of smooth muscle cells [60, 61].

If pathogenesis of atherosclerosis is viewed, in the light of these previously stated facts, as an inflammation, it can be concluded that during the early stages, atherosclerosis, as a specialized form of inflammation, responds to an injury of vascular wall and the presence of different pathogens [51]. With further progression of inflammatory reaction, during the stages of advanced atherosclerotic lesions, development of a chronic inflammatory fibroproliferative response occurs, which is histologically manifested as atheroma (type IV), lesion with a lipid core; fibroatheroma (type V), lesion with a lipid core and prominent fibrous connective tissue above it, or complicated atherosclerotic lesion that is defined as type IV or V lesions with disruptions of lesion surface, hematoma or hemorrhage, and developed thrombotic deposits (Figures 7 and 8) [28, 45, 46, 48].

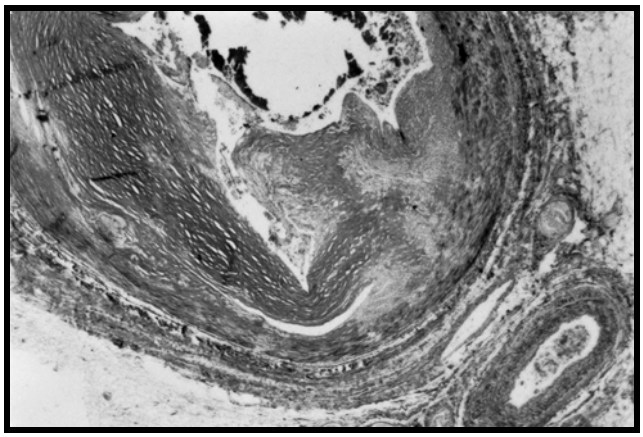

Fig. 7 – Advanced atherosclerotic lesion in the coronary artery – complicated, ulcerated atherosclerotic plaque with intraplaque hemorrhage (histochemical staining of Azzan-Heidenhain, original magnification ×32)

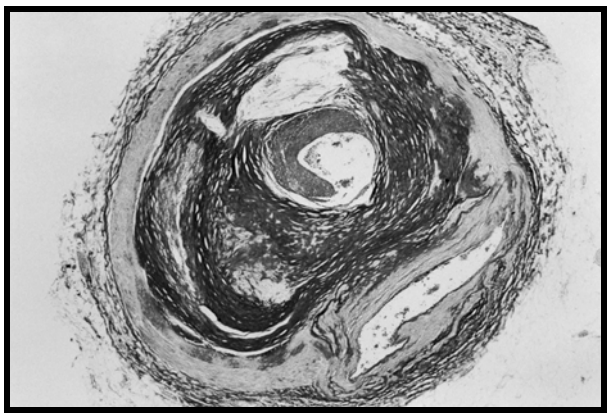

Fig. 8 – Advanced atherosclerotic lesion in the coronary artery – complicated atherosclerotic plaque with atheroma, fibroatheroma, fissure, intraplaque hemorrhage and thrombus (histochemical staining of Azzan-Heidenhain, original magnification ×8)

Conclusion

From all the previously presented facts we can conclude that the basic predisposing factor for development of atherosclerosis is hypercholesterolemia or infection, coupled with systemic hypertension.

The increased levels of plasma cholesterol promote the entrance of lipid droplets in subendothelial layer, their binding to proteoglycans and modification of LDL. Modified LDL stimulates increased expression of adhesion molecules and promotes the adhesion of leukocytes. Modified LDL also stimulates the production of cytokines-chemokines in endothelial cells which promotes the migration of leukocytes into the subendothelial layer, and also initiates synthesis of growth factors which affect differentiation, modulation, migration and proliferation of different cell types in the lesion. From the above presented facts we can conclude, too, that hypercholesterolemia starts *circulus vitiosus* in which many combined factors induce the development of inflammation in atherosclerosis. In addition to modified forms of LDL, the presence of other exogenous pathogens (especially microorganisms) initiates atherosclerosis through inflammatory response of vascular wall.

On the other hand, systemic hypertension promotes vascular remodeling. This process is characterized by smooth muscle cells phenotype modulation from contractile to synthetic phenotype, their migration from media into the subendothelial layer, their proliferation and collagen synthesis, as well as their transformation to foam cells in the presence of modified LDL, inflammatory cytokines and biomechanical strength of blood shear stress.

The previously analyzed facts suggest that atherosclerosis could be defined as inflammation which is a response to hypercholesterolemia or other exogenous pathogens, coupled with vascular remodeling, caused by systemic hypertension.

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A K T U E L N A T E M A

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Virusne infekcije i oksidativni stres

Viral infections and oxidative stress

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Uvod

Prošlo je više od pola veka od prve publikacije na temu toksičnog efekta slobodnih kiseoničnih radikala. Gerschman i sar. [1] su 1954. godine prvi objavili rad u kome su izneli da je toksičnost kiseonika posledica štetnog dejstva slobodnih radikala kiseonika. Dve godine kasnije Harman i sar. [2] izneli su tezu prema kojoj oksidativni stres predstavlja osnovni mehanizam procesa starenja i time pokreću opsežna istraživanja slobodnih radikala, inspirisana idejama večne mladosti i dobrog zdravlja. Sledeće veliko otkriće u ovom području bilo je vezano za identifikaciju jednog od najznačajnijih enzima u živim sistemima, superoksid dismutaze (SOD), koji je potvrdio veliki značaj oskidativnih procesa u živim sistemima. Treća era proučavanja slobodnih radikala počela je 1977. kada su Mital i Murad otkrili da su se živi sistemi ne samo donekle prilagodili dejstvu slobodnih radikala, već ih i iskoristili za sopstveno efikasnije funkcionisanje. Ovaj par naučnika dao je ozbiljne dokaze da hidroksilni slobodni radikal ($^{\bullet}OH$) prenosi signale unutar ćelije stimulišući sintezu sekundarnog glasnika cikličnog guanozin monofosfata (cGMP) [4].

Stvaranje slobodnih radikala je fiziološki proces. Tokom normalnog metabolizma ćelije stvaraju se slobodni kiseonični (*reactive oxygen species* – ROS) i azotni radikali (*reactive nitrogen species* – RNS) koji mogu imati i pozitivne i negativne efekte na živi sistem [5]. U niskim ili srednjim koncentracijama ROS i RNS ostvaruju svoju fiziološku ulogu zaštite ćelije od različitih štetnih uticaja, kao i ulogu u unutarćelijskoj komunikaciji. Međutim, kada produkcija slobodnih radikala premaši određenu koncentraciju i kada se time naruši redoks potencijal ćelije, ispoljava se njihov negativni efekat, odnosno dolazi do oksidativnog ili nitrozativnog stresa [6, 7]. Usled povećanog stvaranja slobodnih radikala, smanjene raspoloživosti antioksidanasa i/ili povećane potrošnje aktioksidanasa dolazi do poremećaja „redoks homeostaze“. Ovako stvoreni „višak“ ROS, odnosno oksidativni stres, imaće za posledicu oštećenje ćelijskih lipida, proteina i nukleinskih kiselina [8, 9].

Ove činjenice bile su osnov velikog broja istraživanja koji su oksidativni stres naveli kao osnovni patogenetski mehanizam nastanka bolesti i procesa starenja.

Slobodni kiseonični radikali

Slobodni radikali mogu se definisati kao molekuli ili delovi molekula koji imaju jedan ili više slobodnih elektrona u atomskim ili molekularnim orbitama što ih čini veoma reaktivnim [7]. Najvažniji slobodni radikali u živim sistemima su slobodni kiseonični radikali (ROS). Primarni ROS je superoksidni anjon ($O_2^{\bullet-}$), koji u daljim reakcijama sa molekulima stvara druge slobodne radikale. Superoksidni anjon najvećim delom stvara se u mitohondrijskom respiratornom lancu i pretpostavka je da oko 1-3% kiseonika „iscuri“ iz ovog lanca kao $O_2^{\bullet-}$, umesto da doprinese redukciji kiseonika. Redukcija superoksida do vodonik-peroksida odvija se pomoću enzima superoksid dismutaze (SOD). Izoenzimi SOD nalaze se u mitohondrijama (mitohondrijska SOD – MnSOD), citosolu (citosolna SOD – CuZnSOD) i na ćelijskim površinama. Slobodni kiseonički radikali imaju veoma važnu ulogu u održavanju redoks potencijala ćelije.

Prisustvo veće koncetracije $O_2^{\bullet-}$ stimuliše stvaranje hidroksil radikala ($^{\bullet}OH$) reakcijom sa vodonik-peroksidom ili oksidacijom gvožđa. U prisustvu povećane količine slobodnog intracelularnog gvožđa (hronični hepatitis C), povećano je i stvaranje slobodnih radikala. Hidroksilni radikal ima veoma kratak poluživot od svega 10^{-9} sekundi, te je odgovoran za oštećenje okolnih struktura – DNK i masnih kiselina. Trajno oksidativno oštećenje DNK predstavlja prvi korak u mutagenezi, karcinogenezi i starenju [4].

Superoksidni anjon u reakciji sa polinezasićenim masnim kiselinama fosfolipida dovodi do stvaranja hidroperoksil radikala. Ovim započinje proces lipidne peroksidacije, a za-

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vršava se stvaranjem malonildialdehida (MDA) koji izaziva mutagenezu, ali je i baktericidan [10].

Usled oksidativnog stresa dolazi i do oksidacije proteina sa većim brojem različitih reakcija (hidroksilacije aromatičnih grupa i alifatičnih bočnih lanaca aminokiselina, nitracije ostataka aromatičnih aminokiselina, sulfoksidacije metionin ostataka, konverzije aminokiselinskih ostataka u karbonil derivate). Treba napomenuti da su metioninski i cisteinski ostaci najosetljiviji na sve tipove ROS-a. Ipak, za razliku od drugih vrsta oksidacija, oksidacija aminokiselina koje sadrže SH grupu je reverzibilna. Kako najveći broj mehanizama oksidacije dovodi do stvaranja karbonil derivata, ova grupa se i najčešće koristi kao marker oksidativnog oštećenja proteina [11, 12].

U živim sistemima postoje brojni odbrambeni mehanizmi usmereni ka održanju homeostaze ćelije i sprečavanju pojave oksidativnog stresa. Antioksidativna odbrana obuhvata neutralizaciju slobodnih radikala, mehanizme oporavka od oštećenja i razvijen antioksidativni sistem. Organizam poseduje niz enzimskih i neenzimskih antioksidanasa. Najvažniji enzimski antioksidansi su superoksid dismutaza (SOD), glutation peroksidaza (GPx), glutation reduktaza (GR), glutation-S-transferaza (GST) i katalaza (CAT), a od neenzimskih glutation i koenzim Q. Egzogeni antioksidansi su vitamini C i E, karotinoidi i flavonoidi. Mogućnosti suplementacije antioksidansima u cilju sprečavanja oksidativnog stresa, a time mnogih bolesti i starenja, danas su fokus brojnih istraživanja.

Od navedenih antioksidanasa, glutation ima posebno važnu ulogu, naročito u virusnim infekcijama. Tako, na primer, virus HIV-a direktno utiče na metabolizam ovog antioksidansa, što za posledicu ima ozbiljan poremećaj „redoks homeostaze“ i konačno oksidativni stres. Glutation je kofaktor nekoliko antioksidativnih enzima, utiče na transport aminokiselina kroz membranu i neutrališe hidroksilne radikale, obnavlja vitamine C i E. Nivo glutationa u ćeliji, preciznije odnos redukovanog i oksidovanog glutationa, dobar je pokazatelj redoks stanja ćelije [13, 14].

Tokom ćelijskog ciklusa postoji stalna fluktuacija redoks potencijala koju u znatnoj meri reguliše glutation. Ove male oscilacije imaju svoje fiziološke funkcije. Manja dominacija redukujuće sredine stimuliše proliferaciju ćelija. Sa druge strane, mali pomak ka oksidativnoj sredini stimuliše diferencijaciju ćelija u kojoj ROS imaju ulogu sekundarnog glasnika. Dalji pomak ka oksidativnijoj sredini dovodi do apoptoze, a teži poremećaj redoks homeostaze do nekroze ćelije [8].

Slobodni kiseonični radikali, svakako, imaju značajnu ulogu u infekcijama. Oni, kao jedan od najstarijih i najefikasnijih oružja imunog sistema, predstavljaju prvu liniju odbrane od infektivnih agenasa. Do oksidativnog stresa dolazi u toku infekcija virusima, bakterijama, rikecijama i parazitima [15, 16]. Kao primarni ROS nastaje $O_2^{\cdot-}$ koji dalje stimuliše stvaranje ostalih slobodnih radikala.

Inflamatorne ćelije su glavni izvor ROS, mada se slobodni radikali mogu stvarati u svim ćelijama. Uslov stvaranja ROS u ovim ćelijama je reakcija receptora i liganda. Ligandi mogu biti virusni i bakterijski antigeni. Kao odgovor na mikrobne antigene, fagocitne ćelije, neutrofili, monociti i makrofagi, dovode do nastanka „respiratornog praska“. Naime, po vezivanju liganda za receptor dolazi do aktivacije sekundarnog glasnika i protein kinaze C koji dovode do translokacije redukovanog oblika nikotinamid-dinukleotid-fosfata (NADPH) u područje plazma membrane. On je glavni izvor slobodnih radikala tokom respiratornog praska. Slobodni kiseonični radikali izlučuju se van ćelije i oštećuju okolne zdrave ćelije. Intracelularni ROS stvoreni su kao nespecifična odbrana i indukuju uništavanje mikroorganizama zarobljenih u fagolizozomima. Unutar ćelije ROS funkcionišu i kao sekundarni glasnici. Oni aktiviraju faktore transkripcije, stimulišu stvaranje citokina i hemokina koji aktiviraju druge inflamatorne ćelije. Povratno, tumor nekrozis faktor (TNF), interleukini IL-1 i IL-6, kao inflamatorni medijatori, mogu da indukuju stvaranje ROS [15–17]. Slobodni kiseonični radikali oslobođeni tokom imunološke reakcije doprinose borbi protiv infekcije direktnim toksičnim efektom, ali i aktivacijom ćelijskog imuniteta preko aktivacije T-ćelija i zato su važna veza nespecifičnog urođenog i stečenog celularnog imuniteta [18].

Hronične virusne infekcije

Početak intenzivnijeg istraživanja oksidativnog stresa tokom infekcija vezan je za proučavanje patogeneze HIV infekcije. Interesovanje je pobudio izveštaj Mullera i sar. [19] o smanjenoj koncentraciji glutationa u limfocitima i drugim ćelijama inficiranim HIV-om. U početku nije bilo jasno da li je smanjenje koncentracije glutationa posledica direktnog dejstva na metabolizam antioksidansa, disfunkcije makrofaga ili povećanog stvaranja ROS i time povećane potrošnje glutationa.

Opšti patogenetski mehanizmi vezani za stvaranje ROS i infekciju indentični su za sve viruse. Virusi dovode do poremećaja redoks homeostaze unutar inficirane ćelije. Pored toga, oni dovode i do povećanog stvaranja ROS u aktiviranim fagocitima [15].

Po ulasku u ćeliju virusi započinju svoj intracelularni parazitski ciklus koristeći resurse domaćina za sopstvenu replikaciju, a po cenu poremećaja biohemijskih procesa unutar ćelije. U mitohondrijama i endoplazmatskom retikulumu ćelije dolazi do značajnog povećanja produkcije slobodnih radikala i posledične potrošnje antioksidantnih potencijala ćelije. Slobodni kiseonični radikali započinju ciklus lipidne peroksidacije i oštećenja DNK. Poremećaj redoks stanja dovodi do apoptoze inficirane ćelije. Pokazano je da intenzitet apoptoze i lize ćelija korelišu sa intenzitetom oksidativnog stresa [18–20].

Specifičniji patogenetski mehanizmi zavise, naravno, i od karakteristika virusa.

Dosadašnja istraživanja virusnih infekcija i oksidativnog stresa bila su usmerena prema određenim virusima koje ćemo u nastavku detaljnije opisati, a to su: infekcija HIV-om, hepatitis C virusom (HCV) i Epstein Barr virusom (EBV).

HIV infekcija

Studije vezane za patogenezu HIV infekcije bile su među prvim koje su detaljnije istraživale vezu infekcije i oksi-

dativnog stresa. Kako i kod drugih infektivnih agenasa, tako i u slučaju HIV infekcije, virusni antigeni su pokretači stvaranja ROS. Studije su pokazale da gp120, gp 24 i tat protein indukuju stvaranje ROS u inflamatornim ćelijama [15, 21, 22].

Oksidativni stres igra veoma važnu ulogu u patogenezi HIV infekcije zbog toga što izaziva apoptozu limfocita. Tokom oksidativnog stresa dolazi i do supresije limfocitogeneze, a smanjen broj limfocita ubrzava progresiju bolesti. Nekoliko istraživanja pokazalo je da je kod bolesnika sa HIV-om smanjena koncetracija antioksidanasa [23, 24].

Značaj oksidativnog stresa tokom HIV infekcije još nije razjašnjen. Novija istraživanja HIV infekcije i oksidativnog stresa uglavnom su usmerena ka izučavanju patogeneze nastanka HIV encefalopatije, odnosno HIV demencije [25–27]. Demencija u sklopu HIV infekcije obuhvata spektar kliničkih manifestacija od blagog kognitivnog poremećaja do teških neuropsihijatrijskih poremećaja. Neurološki deficiti posledica su neuronske degeneracije, gubitka sinapsi i neuronske smrti. Zanimljivo je da su neuroni vrlo retko inficirani virusom, a da do degeneracije dolazi dejstvom slobodnih radikala u toku oksidativnog stresa [27]. Usled dejstva gp 120 i tat proteina HIV-a dolazi do značajnog oksidativnog stresa u neuronima, ali ne i u astrogliji. Razlog je činjenica da astrociti imaju mogućnost povećavanja aktivnosti mangan SOD (MnSOD), glavnog antioksidansnog enzima. Neuroni imaju početno manju koncentraciju MnSOD i mnogo manju mogućnost povećanja aktivnosti ovog enzima [28].

Savremena terapija HIV infekcije (*highly active antiretroviral therapy* - HAART) daleko je od savršenstva. Ipak, činjenica jeste da je bolesnicima na HAART-u očekivana dužina života praktično indentična osobama koje su HIV negativne, ukoliko se terapija započne na vreme i redovno uzima. Glavni neželjeni efekti terapije jesu metaboličke promene od kojih dislipidemija sa posledičnom aterosklerozom zauzima prvo mesto [29].

Još uvek nije jasno da li HAART izaziva oksidativni stres. U zanimljivom istraživanju Hulgan i sar. [29] našli su da su markeri lipidne peroksidacije povećani kod bolesnika sa manjom količinom virusa u krvi, nevezano sa hiperlipidemijom. Autori pretpostavljaju da je oksidativni stres nastao u sklopu imunorekonstruktivnog sindroma. U istom istraživanju lipidna peroksidacija korelisala je sa prisustvom efavirensa u HAART režimu. Ipak, radi se o istraživanju sa relativno malo ispitanika bez kontrole komplijanse i bez određene dužine trajanja terapije. S obzirom na to da je lipidna peroksidacija vezana za pojavu i razvoj ateroskleroze, ovim istraživanjem, svakako, pokrenuta su važna pitanja u smislu toksičnosti antivirusne terapije [29].

Hepatitis C virusna infekcija

Oksidativni stres igra veoma važnu ulogu u oštećenju jetre bilo koje etiologije. Iako je patofiziologija hepatitisa kompleksna, postoji veliki broj dokaza da oksidativni stres igra važnu ulogu u perzistiranju infekcije, progresiji nekroze i fibroze, pa čak i kancerogenezi [30].

Kod dece sa hroničnim hepatitisom B i C primećena je smanjena aktivnost katalaze i SOD, a povećana lipidna peroksidacija ukazuje na postojanje neadekvatnog antioksidativnog odgovora [22].

Zanimljivo, oksidativni stres više je izražen pri infekciji HCV nego hepatitis B virusom. Kao mogući uzrok ovome navodi se opterećenje gvožđem specifično za infekciju HCV-om [31].

Bolesnici sa hroničnom hepatitis C virusnom infekcijom imaju povećan nivo markera lipidne peroksidacije u serumu, perifernim mononuklearnim ćelijama i uzorcima jetre [32, 33]. Čak i kod pacijenata bez simptoma pokazano je da fluktuacija alanin-aminotransferaza koreliše sa poremećajima redoks homeostaze [34–37], što, svakako, pokazuje značaj oksidativnog stresa u patogenezi hroničnog hepatitisa C. Vidali i sar. [38] dokazali su prisustvo markera oksidativnog stresa kod 61% bolesnika obolelih od hroničnog hepatitisa C.

Različiti mehanizmi dovode do povećanog oksidativnog stresa u slučaju HCV infekcije. Hronična inflamacija i aktivacija fagocita su nespecifičan patogenski mehanizam, zajednički mnogim hroničnim infekcijama. Nagomilavanje gvožđa unutar hepatocita stimuliše stvaranje hidroksil radikala koji, dalje, pokreće lipidnu peroksidaciju. Pored oskidativnog stresa koji se dešava u samim hepatocitima, dolazi i do aktivacije susednih Kupferovih ćelija u kojima je povećana sinteza citokina koji dalje povećavaju nivo ROS [36].

Ireverzibilna oštećenja ćelije i poremećaj unutarćelijskog prenosa signala mogu biti izazvani oksidativnim stresom usled HCV infekcije. Kao posledicu delovanja ROS spominju se razvoj steatoze, fibroze, hepatocelularnog karcinoma i B-ćelijskog limfoma [35, 37, 38].

Povezanost oksidativnog stresa i steatoze jetre uslovljena je tipom HCV. Među bolesnicima sa genotipom 3 ova korelacija nije nađena. Sa druge strane, kod bolesnika sa „non-3“ genotipom oksidativno oštećenje može biti uzrok nastanka „metaboličke“ steatoze u kojoj stepen steatoze korelira sa stepenom oskidativnog stresa. Ustanovljena je povezanost degradacije apolipoproteina B100 sa poremećajem sekrecije lipoproteina veoma niske gustine (*very low-density lipoprotein* – VLDL). Drugi uzrok može biti oksidacija slobodnih masnih kiselina koja se dešava pri stvaranju i delovanju ROS. Insulinska rezistenicija kao posledica oksidativnog stresa, takođe, doprinosi razvoju steatoze [35, 39].

Nepoznanica je još uvek to koliko oksidativni stres pri hroničnoj HCV infekciji doprinosi fibrozi jetre. Određena istraživanja pokazuju direktnu povezanost, dok druga ukazuju na njihovu indirektnu povezanost, preko steatoze jetre [35, 40].

Hepatitis C virus povezuje se sa kancerogenezom, a kao jedan od hipotetičkih patogenetskih mehanizama navodi se i oksidativni stres. Poznato je da delovanjem ROS na DNA nastaju mutacije. U slučaju HCV infekcije mogući genotoksični efekti dokazani su *in vitro* i *in vivo*, a manifestuju se u segmentima tumor supresor gena i protoonkogena [37, 40].

Epstein Barr virusna infekcija

Primarna infekcija EBV dešava se uglavnom asimptomatski, tokom detinjstva i kao svaka herpes virusna infekcija dovodi do uspostavljanja latentne infekcije. Ciljne ćelije ovog virusa pretežno su B limfociti jer sadrže najviše

CD21 receptora, ali i druge ćelije, npr. nazofaringealnog epitela, mogu biti inficirane [41]. Kao i u slučaju drugih infekcija, prisustvo oksidativnog stresa je posledica nespecifičnog imunog odgovora organizma. Merenjem imunoloških parametara lipidne peroksidacije (MDA) utvrđeno je da do stvaranja ROS dolazi već nakon 2 sata od infekcije i da se njihova koncentracija povećava tokom 24 sata. Glavni antioksidans u slučaju EBV infekcije jesu katalaza i SOD čija se aktivnost povećava proporcionalno povećanju koncentracije ROS [42].

Epstein Bar virusna infekcija povezuje se sa limfoproliferativnim bolestima kao što je Burkittov limfom, nazofaringealni karcinom i karcinom želuca. Onkogeni potencijal EBV objašnjava se uticajem virusa na signalne procese unutar ćelije domaćina [43, 44]. Pored toga, povećano stvaranje ROS utvrđeno je u ćelijskim linijama porekla tumorskog tkiva pozitivnih na EBV [42]. Za sada, istraživanja su bila pretežno fokusirana na odnos oksidativnog stresa i nazofaringealnog karcinoma i oksidativnog stresa i Burkittovog limfoma [45]. Mehanizam karcinogeneze u slučaju latentne ili retko perzistentne EBV infekcije je višestruk [46]. Lo i sar. [47] pretpostavljaju da je „oksidativna karcinogeneza" posledica aktivacije transkripcijskih proteina (STAT3, NK-kB, MAPK).

Drugi mehanizam može biti smanjenje aktivnosti SOD *in vivo* u slučaju nazofaringelanog karcinoma [1]. Superoksid dismutaza sada se smatra tumor supresorskim proteinom. Zanimljivo je da tokom infekcije EBV dolazi do pojave autoantitela na izoenzim MnSOD (SOD2) koja *in vitro* i *in vivo* smanjuje aktivnost MnSOD. Može se pretpostaviti da blokadom antioksidansa tokom EBV infekcije dolazi do akumulacije superoksida, prekusora ostalih ROS, što dovodi do poremećaja endotelne funkcije i doprinosi patomorfološkim promenama kao što su edem, splenomegalija, mikrokrvarenja i hepatitis [48].

Sindrom hroničnog umora (*chronic fatigue syndrome* - CFS), kao entitet koji se vezuje za infekciju EBV, relativno je čest poremećaj. Definiše se kao stanje perzistirajućeg teškog onesposobljavajućeg umora koji bolesnike sprečava da obavljaju svoje svakodnevne radne, lične i društvene obaveze [49–52]. Tačni etiološki faktori CFS-a nisu još u potpunosti identifikovani, ali virusne infekcije i oksidativni stres izazvan virusnom infekcijom ostaju jedan od najverovatnijih patogenetskih mehanizama [53, 54].

Druge infekcije

Oksidativni stres ispitivan je i u nekim drugim infekcijama koje se povezuju sa karcinogenezom, kao što su infekcija humanim papiloma virusom i bakterijom *Helicobater pylori*. Istraživanja su pokazala akumulaciju ROS u gastričnoj mukozi inficiranih osoba. Nivo oksidativnog stresa korelisao je sa stepenom oštećenja DNK, a u epitelnim ćelijama dokazana je i povećana ekspresija gena koji kodiraju važan DNK reparatorni enzim [55]. Pretpostavlja se da ROS stvara sama bakterija, ali i fagociti na mestu infekcije, a da potom dolazi do ubrzane apoptoze i karcinogeneze [55, 56]. Hrana bogata svežim voćem i povrćem kao i suplemenatcija antioksidansima, posebno vitaminom C, smanjuje učestalost pojave karcinoma želuca [57].

Više od decenije izvode se detaljna ispitivanja sa ciljem utvrđivanja veze hronične inflamacije izazvane humanim papiloma virusom (HPV) i raka grlića materice. Hronična inflamacija, kao i u slučaju hepatitisa C, mogući je patogenetski mehanizam koji dovodi do poremećaja apoptoze i oštećenja genetskog materijala. U slučaju HPV infekcije, identifikovani su humani proonkogeni koji se aktiviraju oštećenjem DNK, nastalih u uslovima oksidativnog stresa, a koji dovode do displazije [58].

Zanimljivo je da je marker oksidativnog ošetećenja DNK 8-nitroguanin, nađen kod neoplastičnih lezija cerviksa izazvanih HPV, ali i kod hepatocelularnog karcinoma nastalog usled hronične hepatitis C virusne infekcije i kod displastičnih ćelija epitela grastrične mukoze u slučaju *Helicobacter pylori* infekcije [59, 60].

Antioksidansi u prevenciji i terapiji infekcija

S obzirom na to da oksidativni stres može da dovede do ozbiljnih hroničnih oboljenja, čak i do karcinoma, postavlja se pitanje opravdanosti suplemenatcije antioksidansima. Mnoge studije o oksidativnom stresu i infekcijama pokrenule su još veća istraživanja na temu antioksidantne suplementacije. Pregled literature o tome, svakako, zaslužuje poseban pregledni članak, ali bi neke osnovne činjenice trebalo izneti umesto zaključka, s obzirom na široko rasprostranjenu upotrebu antioksidantnih suplemenata.

Pregledom literature jasno je da je najbolje proučeni suplement vitamin C. Ozbiljni pregledni članci kao oni iz *Cochrane* biblioteke zaključuju da zaista postoji povoljan efekat u smislu prevencije respiratornih virusnih infekcija, ali da je suplementacija vitaminom C veća od 0,2 g opravdana samo kod ljudi koji su izloženi većem fizičkom stresu, nepravilnom načinu ishrane, stalnoj izloženosti toksičnim noksama (pušenje) ili izraženoj hladnoći [61, 62].

Suplementacija selenom pokazala se bitnom u prevenciji *Coxsackie* infekcija, dok su se selen i glutation pokazali efikasnim u prevenciji infekcija izazvanih virusima *Influenzae* [63, 64]. U slučaju težih infekcija i sepse selen i glutamin su pokazali određene kliničke efekte [65]. Oba su povezana sa enzimom glutation peroksidazom, koji jeste jedan od najznačajnijih antioksidantnih enzima [66–70].

Prevencija karcinoma uslovljenih hroničnim infekcijama ispitana je u velikom broju studija. Pokazano je da dijetalni antoksidansi imaju veoma ograničen efekat na prevenciju oštećenja DNK. Pored velikog broja istraživanja, mali broj njih urađen je po principima medicine zasnovane na dokazima, ali su rezultati ukazali da je ukupan efekat antioksidanasa bio manji nego očekivani. Čini se da je pravilna ishrana bogata antioksidansima efikasnija od suplementacije. Ovo se posebno odnosi na populaciju sa, inače, lošim načinom ishrane, što je nađeno u nekoliko studija.

I pored zaista velikog broja originalnih studija, metaanaliza i preglednih članaka, ne postoji jasan konsenzus o pitanju antioksidantne suplementacije tokom infekcija. Postoji stvarna potreba za dobro kontrolisanim studijama sa jasno definisanim ciljevima. Oksidativni stres tokom infekcija je realno stanje, a mogućnosti suplemenatcije antioksidansima su realno pitanje na koje odgovor tek sledi.

Zaključak

Dosadašnja istraživanja na temu virusnih infekcija i oksidativnog stresa odnose se pretežno na infekcije herpes virusima (EBV), primarnim hepatotropnim virusima (virusima hepatitisa C i hepatitisa B) i HIV infekciju. Cilj i značaj razjašnjenja uticaja oksidativnog stresa tokom ovih infekcija jeste poboljšanje terapije i potencijalna prevencija virusnih infekcija. Po pitanju antioksidantne suplementacije tokom infekcija, ne postoji zvaničan medicinski konsenzus i postoji stvarna potreba za dobro kontrolisanim istraživanjima koja će nam dati konačne odgovore na ovu temu.

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Citohemijske i imunocitohemijske karakteristike Mekelovog divertikuluma sa heterotopičnim pankreasnim tkivom

Cytochemical and immunocytochemical characteristics of Meckel's diverticulum with heterotopic rests of pancreatic tissue

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Apstrakt

Uvod. Mekelov divertikulum (MD) je kongenitalna anomalija tankog creva. Nastaje zbog nepotpune obliteracije i resorpcije proksimalnog dela omfaloenteričnog kanala koji povezuje žumančanu kesu sa primitivnim crevom u fetalnom periodu. **Prikaz bolesnika.** Prikazana je dvadesetogodišnja devojka sa ektopičnim tkivom pankreasa u MD. Hospitalizovana je sa kliničkim znacima akutnog apendicitisa. Tokom operacije nađen je inflamirani MD i urađena je klinasta resekcija divertikuluma. Patohistološki nalaz otkrio je pankreasno tkivo u uklonjenom divertikulumu. Endokrine ćelije (EC) dokazane su Massonovim bojenjem, a aberantno pankreasno tkivo imunocitohemijskom metodom LSAB2, uz upotrebu epitelnog markera pan citokeratina. **Zaključak.** Većina MD je asimtomatska i otkriva se pri laparotomiji zbog drugih razloga, ali komplikacije nedijagnistkovanog MD mogu biti ozbiljne (divertikulitis, perforacija sa peritonitisom ili intestinalna opstrukcija zbog invaginacije). U nejasnim slučajevima, dijagnostika se može sprovesti korišćenjem dodatnih citohemijskih ili imunohistohemijskih metoda.

Ključne reči:
mekelov divertikulum; dijagnostika; histocitohemija; imunohistohemija; pankreas; horistoma.

Abstract

Background. Meckel's diverticulum (MD) is a congenital anomaly of the small intestine. It results from incomplete obliteration and resorption of the proximal omphaloenteric duct connecting yolk sac with primitive gut in the fetal period. **Case report.** A case of 20-year old female with ectopic pancreatic rests in a MD was reported. She was hospitalized with clinical signs of acute appendicitis. During surgery an inflamated Meckel's diverticulum was found and a clinoid resection of the diverticulum was performed. Histologic examination revealed pancreatic tissue in the removed diverticulum. Endocrine cells (EC) were detected with Masson staining and aberrant pancreatic tissue with immunocytochemical LSAB2 method using pan cytokeratin as epithelial marker. **Conclusion.** Most of MD are asymptomatic and accessory finding during laparothomias for different causes, but complications of undiagnozed MD can be serious (diverticulitis, perforation with peritonitis or intestinal obstruction caused by invagination). In unclear cases, additional cytochemical and immunocytochemical diagnostics could be done.

Key words:
meckel diverticulum; diagnostic tecniques, digestive system; histocytochemistry; immunohistochemistry; pancreas; choristoma.

Uvod

Mekelov divertikulum (MD) najčešća je i klinički najvažnija kongenitalna anomalija tankog creva. Nastaje zbog izostanka potpune obliteracije omfaloenteričnog kanala [1]. Javlja se po „pravilu dvojke" koje se može naći u većini klasičnih udžbenika patologije: sreće se kod 2% populacije, dva puta je češći kod muškaraca, najčešće se nalazi kod mlađih od dve godine i najčešće na dve stope (30,48 cm) od ileocekalnog spoja [2]. Dve trećine bolesnika mlađi su od dve godine. Divertikulum se nalazi na antimezenteričnoj ivici ileuma, kod odraslih udaljen 60 do 100 cm od ušća ileuma u cekum. Dug je prosečno oko 5 cm, različitog promera, nekada širok koliko i sam ileum i uvek značajno širi od apendiksa. Sa vrha divertikuluma može slobodno da visi fibrozna traka ili da bude pričvršćena drugim krajem za umbilikus [3]. Opisane su i fistule između Mekelovog divertikuluma i umbilikusa [4, 5]. Većina MD je asimptomatska i otkriva se slučajno pri lapa-

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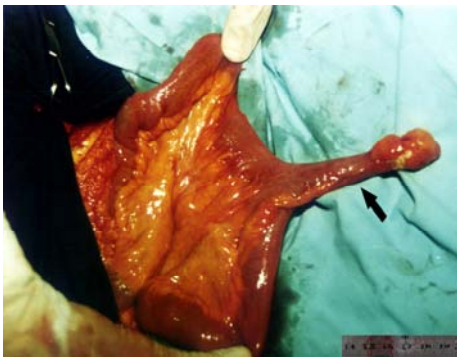
ratomiji zbog nekih drugih razloga, ili na autopsiji [2]. Mekelov divertikulum je pravi divertikulum jer nastaje invaginacijom zida tankog creva, pa sadrži sve njegove slojeve. Sluzokoža divertikuluma slična je sluzokoži susednog dela ileuma. Oko polovina divertikuluma sadrži ostrvca ektopične, funkcionalno aktivne želudačne mukoze koja može izazvati ulceracije i misteriozna intestinalna krvarenja ili simptome koji podsećaju na apendicitis. Manji broj simptomatskih divertikuluma sadrži pankreasno ektopično tkivo. Duodenalno, bilijarno ili tkivo kolona, takođe, mogu da se nađu u divertikulumu [5]. Od ostalih komplikacija javljaju se divertikulitis [6], perforacija sa peritonitisom ili intestinalna opstrukcija zbog invaginacije [7, 8].

Prikaz bolesnika

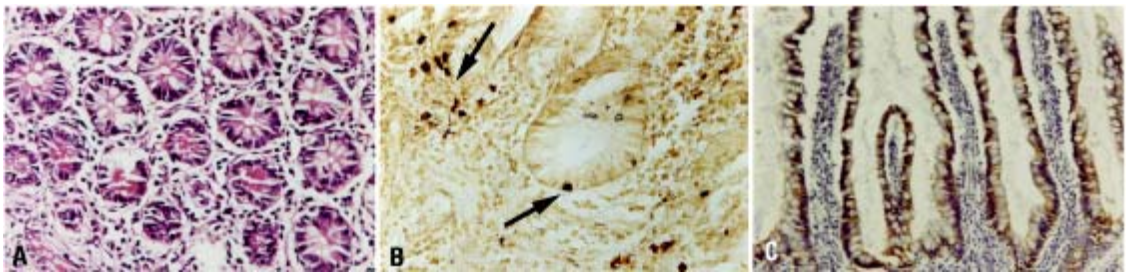
Bolesnica stara 20 godina, hospitalizovana je sa bolom u suprapubičnom delu trbuha. Bol je počeo nekoliko dana ranije u predelu pupka i postepeno se spuštao dole i desno. Zadnja 2–3 dana imala je i mučninu praćenu povraćanjem. Bolesnice nije bila na nekoj specifičnoj terapiji medikamentima, a u istoriji bolesti nije imala podatke o postojanju značajnijih bolesti. Fizikalni pregled pokazao je bolnu osetljivost trbuha u predelu desnog donjeg kvadranta – pozitivan Bloombergov znak. Tri konsekutivna pregleda krvne slike pokazala su leukocitozu (19 000–23 000/mm^3) i normohromnu anemiju. Ostali laboratorijski parametri nisu bili od posebnog značaja. Ultrazvučni pregled abdomena nije pokazivao nikakve specifičnosti. Radiografija trbuha u uspravnom stavu pokazala je znakove subokluzije. Klinička slika i laboratorijski nalazi ukazivali su na apendicitis, pa je bolesnica operisana istog dana po prijemu. Tokom apendektomije otkriven je Mekelov divertikulum na završnom delu ileuma, na 80 cm od ušća ileuma u cekum. Divertikulum se nalazio na antimezenteričnoj ivici tankog creva, bio je cilindričan, širok 2 cm pri bazi i dugačak 5–6 cm, sa znacima inflamacije na vrhu (slika 1). Urađena je klinasta resekcija nađenog MD. Patohistološki nalaz ekstirpiranog tkiva potvrdio je dijagnozu MD i otkrio heterotopno pankreasno tkivo u njemu. Postoperativni tok bolesnice bio je očekivano povoljan, pa je posle nekoliko dana hospitalizacije otpuštena na kućno lečenje.



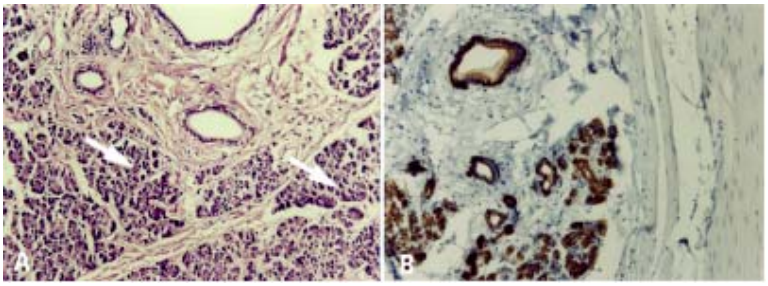
Sl. 1 – Mekelov divertikulum *in situ*

Ekstirpirani MD fiksiran je u 10% formaldehidu, ukalupljen u parafinu i obojen hematoksilin eozionom (HE), Massonovom tehnikom i imunohistohemijskom LSAB2 metodom korišćenjem epitelnog markera, pan citokeratina.

Ekstirpirani MD imao je intestinalni tip mukoze. Bila je prisutna nodularna hiperplazija Liberkinovih žlezda udružena sa hiperplazijom i hipergranulacijom Panetovih (slika 2a) i endokrinih, enterohromafinih ćelija (slika 2b). Pokrovni epitel divertikuluma bio je intestinalnog tipa (slika 2c). Najznačajniji nalaz bio je ektopično pankreasno tkivo koje se, u vidu ostrvaca, difuzno nalazilo na više mesta u MD (slika 3a). Posmatrano u odnosu na slojeve zida, ostrvca pankreasnog tkiva nalazila su se između seroze i mišićnog sloja zida divertikuluma. Radilo se o lobularnom tkivu egzokrinog pankreasa koje se sastojalo od malih acinusa i duktusa i koje



Sl. 2 – Građa Mekelovog divertikuluma: a) hiperplazija Liberkinovih žlezda i Panetovih ćelija (HE ×400); b) hiperplazija endokrinih ćelija sa crnim, argentafinim granulama u citoplazmi (Masson ×400); c) pokrovni epitel intestinalnog tipa u Mekelovom divertikulumu; difuzna citoplazmatska ekspresija (LSAB2 ×200)



Sl. 3 – a) Aberantni pankreas u zidu Mekelovog divertikuluma (HE ×100); b) pankreasno tkivo u zidu Mekelovog divertikuluma (intenzivna, difuzna imunohistohemijska ekspresija pan citokeratina, LSAB2 ×100)

je bilo histološki dokazano primenom monoklonalnog antitela tipa pan citokeratina (epitelni marker – DAKO Copenhagen) (slika 3b).

Diskusija

Mekelov divertikulum najčešći je divertikulum tankog creva. Embrionalno potiče od omfaloenteričnog kanala koji se zatvara u sedmoj gestacijskoj nedelji [1]. Uprkos postojanju i dostupnosti velikog broja dijagnostičkih procedura dijagnoza MD teško se postavlja, zbog čega je nastao iskaz – na MD se često sumnja, još češće se previdi i retko se nađe. Ideja da se prikaže jedan Mekelov divertikulum proizašla je iz želje autora da jasna klinička slika i hronološki tok događaja kod naše bolesnice pomognu da se umanji mogućnost njegovog previđanja. Iako je postavljanje dijagnoze MD najčešće moguće rutinskim patohistološkim tehnikama, u retkim, nejasnim, slučajevima dijagnostika se može sprovesti korišćenjem dodatnih citohemijskih i imunohistohemijskih metoda.

Na osnovu velikog broja istraživanja može se reći da se MD javlja kod 1–4% populacije [9–11], ali i češće [12]. Mekelov divertikulum nalazi se na terminalnom ileumu, kod odraslih osoba na udaljenosti 20–100 cm [5, 10, 13] od ileocekalnog ušća, a kod 90% slučajeva na do 90 cm od samog ušća [8, 14, 15]. Iako se podjednako javlja kod oba pola, MD češće izaziva komplikacije kod muškaraca, pa se zato kod njih češće i dijagnostikuje, na šta ukazuju brojni podaci iz literature [5, 16]. Veća zastupljenost simptomatskog MD kod muškaraca je evidentna, i kreće se od 62 do 80%, u odnosu približno 2 : 1 [10, 17], 3 : 1 [1], ili čak 4 : 1 [15].

Većina Mekelovih divertikuluma ostaje asimptomatska tokom života i pronalazi se slučajno, tokom autopsije, laparatomije ili barijumskih snimanja creva. Velika je šarolikost u literaturi kada se govori o simptomima koje MD može da izazove. Verovatni razlog tome je ciljna grupa bolesnika od koje zavisi klinička slika (uzrast, pol). Generalno je poznato u medicinskoj literaturi da 25% MD postane simptomatsko tokom života, ali neki podaci ukazuju i na veću [15, 17] ili, pak, manju učestalost [1]. Ipak, većina studija navodi da samo 4–6% osoba sa MD nosi rizik da tokom života dobije neke komplikacije [16, 18].

Simptomatski MD klinički se prezentuje kao intestinalna opstrukcija, divertikulitis sa lokalnim ili generalizovanim peritonitisom, ili intestinalna hemoragija, sa ili bez perforacije tankog creva. Klinička prezentacija i incidencija komplikacija koje dovode do operacije Mekelovog divertikuluma različite su i zavise od starosti bolesnika. U pedijatrijskoj populaciji najčešća komplikacija je opstrukcija, dok se kod starije dece i odraslih krvarenje javlja kao najčešća komplikacija [1, 4], mada ima i oprečnih podataka [18]. Divertikulitis Mekeli treća je po učestastalosti komplikacija MD i češće se javlja u starijoj populaciji [5].

Polovina MD sadrži ektopično tkivo koje se najčešće otkriva slučajno u toku laparotomija ili radiografija, kao izrasline koje neznatno štrče u lumen, pa se ponekad zamenjuju sa mnogo ozbiljnijim tumorima. Najčešće se radi o želudačnom, pankreasnom ili jejunalnom ektopičnom tkivu, ali može da se nađe i tkivo kolona, pa čak i ektopični endometrijum. Retko, ektopično tkivo može da predstavlja duodenalno tkivo sa Brunerovim žlezdama ili žučni epitel [5]. Smatra se da oko 50% slučajeva MD ima neku vrstu ektopičnog tkiva od čega 60–85% ima gastričnu mukozu, a 5–16% pankreasno tkivo [16, 17].

Interesantno je zapažanje da je pankreasno ektopično tkivo podjednako zastupljeno i u grupi simptomatskih i asimptomatskih MD [4]. Simptomi zavise od komplikacija (opstrukcija, ulceracija, invaginacija) do kojih to tkivo dovodi samim svojim prisustvom ili postojećim patološkim procesom [19], uključuju bolove, dijareju, krvarenje, zatim opstrukciju ili ulceraciju aberantnog tkiva, a vrlo retko i nastanak tumora [20]. Pankreasno tkivo u zidu MD može da se nađe u vidu manjih ili većih ostrvaca acinusa i duktusa egzokrinog pankreasa, kao što je bilo u prikazanom slučaju, ali mogu da se nađu i Langerhansova ostrvca endokrinog pankreasa.

Lokalizacija ektopičnog tkiva u MD je različita. Prema Parku i sar. [1] ektopično tkivo najčešće se nalazi u bazi divertikuluma. Neki istraživači, međutim, smatraju da lokalizacija ektopičnog tkiva zavisi od veličine divertikuluma. Tako, Dewandel i sar. [15] opisali su da je kod malih divertikuluma ektopično tkivo bilo lokalizovano u distalnim partijama divertikuluma, dok je kod većih od 1,6 cm ono bilo zastupljemo u gotovo svim delovima MD, što je i u našem prikazu bio slučaj. Ovaj nalaz je u saglasnosti i sa nalazima Mukaia i sar. [21].

Hirurški tretman simptomatskih divertikuluma široko je prihvaćen, ali je lečenje asimptomatskih divertikuluma večita dilema abdominalnih hirurga [22], posebno kada je nađen slučajno, tokom neke druge hirurške intervencije. Odluka o divertikulotomiji u takvim slučajevima prepuštena je hirurgu. Ipak, postavljeni su određeni kriterijumi kada MD treba operisati i to su: bolesnici mlađi od 50 godina, muškog pola, divertikulum duži od 2 cm, ili prisutno ektopično tkivo koje se najčešće povezuje sa simptomatskim divertikulumima. Svaki bolesnik koji ispunjava bar jedan od navedenih uslova trebalo bi da se podvrgne divertikulotomiji [1].

Zaključak

Većina MD je asimtomatska i otkriva se pri laparotomiji zbog drugih razloga, ali komplikacije nedijagnostikovanog MD mogu biti ozbiljne (divertikulitis, perforacija sa peritonitisom ili intestinalna opstrukcija zbog invaginacije). U nejasnim slučajevima, dijagnostika se može sprovesti korišćenjem dodatnih citohemijskih ili imunohistohemijskih metoda.

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C A S E R E P O R T UDC 616.006.44–031.2::617.7

A case of primary peripheral T-cell type Non-Hodgkin lymphoma originating in the iris – clinicopathological findings

Primarni periferni T-ćelijski limfom dužice – kliničkopatološke karakteristike

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Abstract

Background. The ocular adnexal region is the primary localization of extranodal lymphoma in 5% to 15% of all Non-Hodgkin lymphoma. Intraocular lymphoma of T-cell origin is extremely rare and such sites of infiltration have been rarely observed in clinical examination. **Case report.** We presented a 56-year-old man with iris infiltration by primary intraocular peripheral T-cell lymphoma. The patient was in clinical stage I BE and the treatment was initiated according to cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone (CHOP) regimen. When the second course of the therapy was scheduled, the patient developed central nervous system lymphoma infiltration. Although De Angelis regimen was used, 3 months after the diagnosis was established, lethal outcome ensued due to disease progression. **Conclusion.** According to our experience we can conclude that further therapeutical approach to patients with primary intraocular T-cell lymphoma requires modification of conventional treatment regimens. The lower median survival in these patients suggests that the disease may be of more aggressive course.

Key words:
lymphoma, non-hodgkin; iris diseases; diagnosis; neoplasm staging; histological techniques; therapeutics; treatment outcome.

Apstrakt

Uvod. Okularni adneksi su primarna lokalizacija ekstranodalnih limfoma kod 5–15% svih *non*-Hodgkin limfoma. Intraokularni limfom T-ćelijskog porekla je redak i u dosadašnjoj kliničkoj praksi nije zabeleženo mnogo ovakvih slučajeva. **Prikaz bolesnika.** Prikazali smo bolesnika, starog 56 godina, sa infiltracijom dužice primarnim perifernim T-ćelijskim limfomom. Bolesnik je inicijalno bio u I BE kliničkom stadijumu i započeto je lečenje prema ciklofosfamid, hidroksidaunorubicin, onkovin, prednizon (CHOP) terapijskom protokolu. U terminu kada je zakazan drugi terapijski ciklus potvrđena je infiltracija centralnog nervnog sistema. Iako je lečenje nastavljeno prema De Angelis protokolu, došlo je do progresije bolesti i smrtnog ishoda tri meseca nakon postavljanja dijagnoze. **Zaključak.** Na osnovu našeg iskustva možemo da zaključimo da konvencionalni terapijski pristup u lečenju bolesnika sa primarnim intraokularnim T-ćelijskim limfomom zahteva modifikaciju. Niža stopa preživljavanja ovih bolesnika ukazuje na to da bolest može imati agresivniji tok.

Ključne reči:
limfom, nehodžkinov; dužica, bolesti; dijagnoza; neoplazme, određivanje stadijuma; histološke tehnike; lečenje; lečenje, ishod.

Introduction

Neoplasmas may affect the eye orbit as a direct result of metastatic neoplastic infiltration, compression, or circulating antibodies involving paraneoplastic retinal degeneration [1]. Non-Hodgkin lymphoma (NHL) constitute one half of all orbital malignancies and ocular adnexa are the primary extranodal lymphoma localization in 5%–15% of all extranodal NHL [2]. Marginal zone B-cell lymphoma of mucosa associated lymphoid tissue (MALT) is the most common lymphoma category arising in these anatomical structures [3]. Intraocular lymphoma of T-cell origin is extremely rare and these sites of infiltration have rarely been observed on clinical examination.

Lymphomas derived from mature (post-thymic) T-cells and natural killer (NK) cells, referred to as peripheral T-cell lymphomas (PTCL), encompass less than 15% of all NHL [4]. Peripheral T-cell lymphoma, Not Otherwise Specified (PTCL NOS) is the most common and most heterogenous category of PTCL. Presentation is usually nodal but any site can be affected and extranodal involvement is common. The median age of patients is 70 years, and almost 65% have ad-

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vanced, clinical stage IV of the disease [4, 5]. The most commonly used treatment is chemotherapy, the cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone (CHOP) regimen or its variations [6].

We studied clinical and histopathological findings of a patient with a very rare iris infiltration by primary intraocular PTCL NOS.

Case report

A 56-year-old man was admitted at the Institute of Hematology, Clinical Center of Serbia, with diagnosis of PTCL, unspecified type, localized in the iris. The diagnosis was made at the Institute of Ophthalmology in May 2008. The clinical features included hyperemic right eye with white membrane in the front segment. On presentation, physical and laboratory findings were normal without B symptoms (systemic symptoms of fiver, night sweats and weight loss). Virusological and bacterial findings, hemostasis of the chest, X-ray, abdominal ultrasound and bone marrow biopsy were normal. Computerized tomography (CT) scan of the brain and paranasal cavities was without pathological findings.

A biopsy of the right eye was performed. Bioptic samples were analysed according to standard histopathological (hematoxylin-eosin, Giemsa and Gordon Sweet) and immunohistochemical (Dako LSAB 2 HRP) procedures.

Histopathological evaluation of the iris tissue specimen showed diffuse tumor infiltration, composed predominantly of the medium-sized lymphoid cells, and rare single large lymphoid cells with vesicular nuclei, central prominent nucleoli and scant basophilic cytoplasm (Figure 1 A, B). Immunohistochemical studies revealed that tumors cells were LCA+, TdT-, CD20-, CD3+, CD5+weak, CD43+, CD45RO+ and CD30- (Figure 1 C–E). Ki-67 was positive in 60% of the tumor cells (Figure 1 F). There was also an inflammatory polymorphus background with clustered CD20+ small lymphocytes, rare eosinophils, plasma cells and epitheloid histiocytes. The morphologic appearance together with the immunophenotype of the tumor were diagnostic for PTCL.

The patients clinical stage was I BE and chemotherapy (CHOP regimen) was started in June 2008. A month later, at the time for the second cycle of the CHOP regimen, the patient was admitted at our Institute in a generally very poor condition, with a fever (38.4 °C) and neurological symptoms: disorientation and left side hemiparesis. Pathological laboratory findings were mild anemia hemoglobin (Hb) 117 g/L, erythrocyte sedimentation rate (ESR) 28 mm/h, elevated lactic dehydrogenase (LDH) 620 U/L and elevated C-reactive protein (CRP) 9,09 mg/L normal range: 0–3 mg/L. Abdominal ultrasound showed mild hepatomegaly (166 mm). Nuclear magnetic resonance (NMR) of the brain revealed multifocal cortical lesions, lesions of the basal ganglias, predominantly periventricular. Although secondary therapy for central nervous system (CNS) lymphoma localization (De Angelis regimen) was started, the disease had progressed and the patient died 3 months after the diagnosis was established.

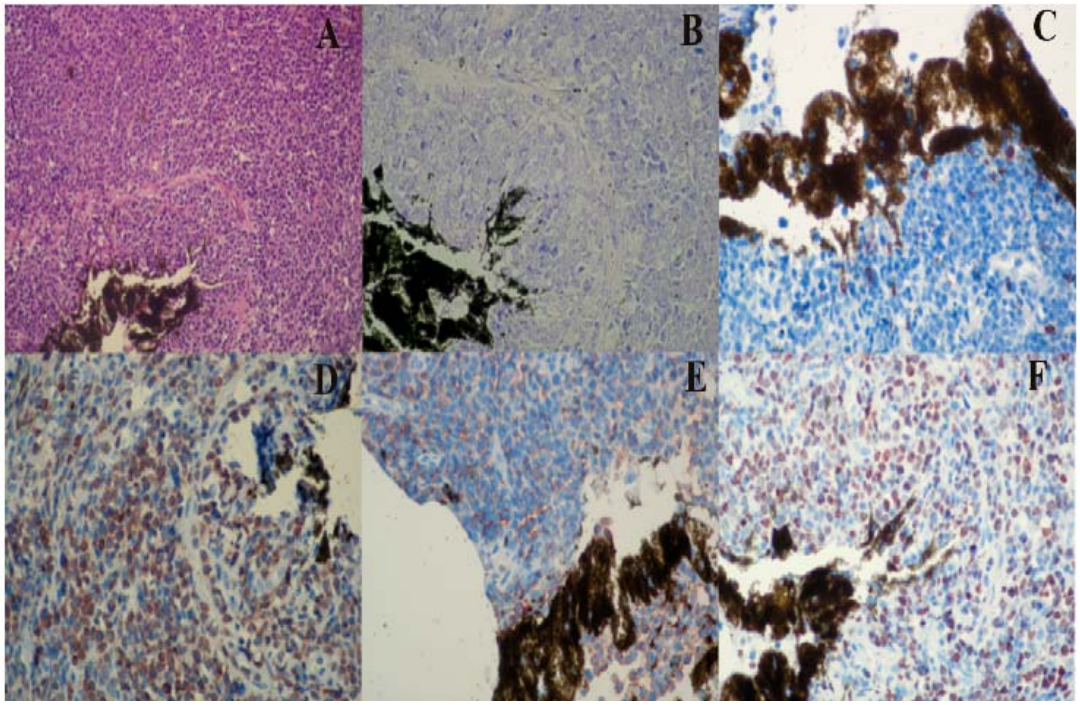

Figure 1 – (A) Iris tissue samples: diffuse tumor infiltration, composed predominantly of the medium-sized lymphoid cells, and rare single large lymphoid cells with vesicular nuclei, central prominent nucleoli and scant basophilic cytoplasm (peripheral T-cell lymphoma; hematoxylin and eosin, ×200); (B) Diffuse tumor infiltration (peripheral T-cell lymphoma; Giemsa, ×400); (C) The tumor cells negative for CD20 (peripheral T-cell lymphoma, ×400); (D) The tumor cells positive for CD3 (peripheral T-cell lymphoma, ×400); (E) The tumor cells positive for CD45RO (peripheral T-cell lymphoma, ×400); (F) The tumor cells positive for Ki67 (peripheral T-cell lymphoma, ×400)

Discussion

Non Hodgkin lymphoma affects ocular tissues either as a primary tumor or as a secondary metastasis from systemic NHL [1]. Intraocular lymphoma is generally of the B-cell type, similar to NHL elsewhere in the body, whereas T-cell type lymphoma is quite rare. The intraocular involvement can be divided into 2 general types [7]. The first is vitreoretinal lymphoma, the most common form, associated with CNS lymphoma, which is usually of the B-cell type. The second is uveal lymphoma, which is associated with visceral or nodal involvement. Between 56% and 85% of patients who initially present with primary intraocular lymphoma alone will develop cerebral lesions [8].

The appropriate diagnosis of ocular NHL can be made on identification of malignant cells in the eye by biopsy, but neuroimaging techniques are fundamental for differential diagnosis, staging and evaluation of therapeutic response. The clinical picture depends on the anatomical sites involved. Usually, there is a slowly growing, painless mass that displaces rather than infiltrates the normal structures, causing an eyelid lump, ptosis or proptosis [9–11]. In our patient, NHL simulated uveitis, and there are some papers describing this as a first NHL sign [12–16]. Some reported cases have been presented as hypopyon uveitis, neovascular glaucoma, diffuse iris thickening or as a lymphomatous lesion [17–19]. Our patient was staged as CSIE with primary iris infiltration, but all published papers of iris lymphoma describe association with systemic NHL, and in one case with primary CNS involvement [15, 18, 19].

Although our patient had no neurological symptoms or signs on presentation, confirmed with normal brain and paranasal cavity CT scan, we speculated whether the iris was the only initial localization because of the very rapid progress to CNS. Lymphoma brain infiltration was confirmed with NMR imaging technique month after the patient started therapy. The routes of infiltration to these specific sites have already been a topic of research [20]. The lymphoma cells enter the brain preferentially through the choroids plexus and cranial nerves. Once within the brain, the cells spread and migrate along the optic nerve sheath into the eyes where they continue to migrate along the choroids, ciliary body, iris, and into the anterior chamber of the eye. The orbit is also infiltrated by the lymphoma cells. However, this occurs independently of the brain-optic nerve-intraocular route.

The therapeutic strategies for this specific localization is controversial due to the fact that the primary lymphoma in the iris, especially of T-cell type, is extremely rare [6, 21]. Orbital lymphomas of MALT type show a better prognosis compared to other lymphoma subtypes arising in the ocular adnexa. Surgical resection, radiotherapy, and alkylating agent-based chemotherapy are the standard approaches for MALT orbital lymphomas [3]. The clinical course of PTCL lymphomas is aggressive, with frequent relapses and poor overall outcomes, using conventional management, with a 5-year overall survival 20%–30% [6]. Many alternate strategies have been assembled based on retrospective data, small case series, single institute experience and phase II studies [21]. Unfortunately, chemotherapy was not sufficient therapeutic approach in our patient, we speculated whether local and CNS prophylactic radiotherapy would have been a better choice. Radiotherapy could normalize the intraocular pressure followed by a reduction in neovascularization of the iris [22].

Conclusion

Presenting our experience with an unusual ocular site of PTCL, we conclude that further therapeutical approach require modification of conventional treatment regimens. The lower median survival in these patients suggests that the disease may be prone to a more aggressive course requiring CNS prophylaxis.

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P R I K A Z S L U Č A J A

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Preoperativna identifikacija mesta krvarenja uzrokovanog angiodisplazijom tankog creva uz pomoć selektivne arteriografije i aplikacije metilenskog plavog

Preoperative identification of bleeding site caused by angiodysplasia of the small bowel by means of selective arteriography and application of methylene blue

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Apstrakt

Uvod. Krvarenja lokalizovana u tankom crevu su retka i javljaju se kod 2–10% svih gastrointestinalnih hemoragija. U slučaju potrebe hirurške intervencije najveći problem predstavlja identifikacija mesta krvarenja. **Prikaz bolesnika.** Prikazan je bolesnik star 65 godina, primljen kao hitan slučaj zbog masivnog gastrointestinalnog krvarenja. Nakon primenjene reanimacije i normalizacije vitalnih parametara, urađena je selektivna arteriografija. Identifikovano je mesto ekstravazacije kontrasta u nivou jejunalnih grana arterije mesenterike superior i obeleženo primenom metilenskog plavog. Neposredno nakon toga izvedena je hirurška intervencija tokom koje je urađena poštedna resekcija obeležene vijuge jejunuma u dužini od 10 cm i terminoterminalna anastomoza. Preparat je poslat na histopatološki pregled kojim je utvrđeno postojanje angiodisplazije tankog creva. Bolesnik je kontrolisan svaka tri meseca, u naredne dve godine, tokom kojih nije bilo epizoda krvarenja. **Zaključak.** Krvarenje uzrokovano angiodisplazijom tankog creva predstavlja značajan dijagnostički problem u slučaju potrebe hitne hirurške intervencije. Kombinovana primena preoperativne selektivne arteriografije i metilenskog plavog omogućava tačnu identifikaciju mesta krvarenja, kao i poštednu resekciju tankog creva čime se izbegava rizik i opasnost od malapsorpcionog sindroma.

Ključne reči:
angiodisplazija; crevo, tanko; angiografija; metilensko plavilo; hirurgija digestivnog sistema, procedure; dijagnoza; lečenje, ishod.

Abstract

Background. Small bowel hemorrhages are rare and account for 2–10% of all gastrointestinal bleedings. In case that surgery is necessary, identification of the bleeding site is the most important problem. **Case report.** We presented here the case of a 65-year old man, admitted for urgent care of massive lower gastrointestinal bleeding. After reanimation and normalization of vital parameters, selective arteriography was done. A contrast extravasation site was identified at the level of jejunal branches of *a. mesenterica superior* and labeled by means of methylene blue application. Immediately after we performed conservative resection of the labeled jejunal loop in 10 cm length and terminoterminal anastomosis. The preparation was sent for histopathologic examination – small bowel angiodysplasia was identified. The patient was monitored in three month intervals in the next two years and new bleeding events were not observed. **Conclusion.** Bleeding caused by small bowel angiodysplasia is a significant diagnostic problem in cases in whom urgent surgery is required. Combined preoperative selective arteriography and methylene blue application make possible precise identification of the bleeding site as well as conservative small bowel surgery, avoiding thus the risk and danger of malabsorption syndrome.

Key words:
angiodisplasia; intestine, small; angiography; methylene blue; digestive system surgical procedures; diagnosis; treatment outcome.

Uvod

Akutna krvarenja iz donjih partija gastrointestinalnog trakta predstavljaju vrlo značajan problem u svakodnevnoj hirurškoj praksi, sa godišnjom incidencijom od 20 do 27 epizoda na 100 000 stanovnika i mortalitetom od 4 do 10% [1]. U slučaju potrebe hitne hirurške intervencije, neophodno je identifikovati mesto krvarenja. Postoje brojne dijagnostičke procedure koje sa različitim stepenom tačnosti mogu utvrditi uzrok i mesto krvarenja. Selektivna arterio-

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grafija predstavlja jednu od najznačajnih metoda identifikacije krvarenja iz gastrointestinalnog trakta uzrokovanog angiodisplazijom. Prema podacima iz literature, arteriografijom je moguće otkriti mesto krvarenja kod preko 80% bolesnika [2]. S obzirom na ishodište krvnih sudova koji vaskularizuju šuplje organe trbušne duplje najčešće se vrši ispitivanje grana abdominalne aorte trunkusa celiakusa gornje i donje mezenterične arterije. Radi poboljšanja preciznosti identifikacije mesta krvarenja moguće je kroz već postavljeni kateter aplikovati metilensko plavo i time jasno ograničiti mesto buduće resekcije određenog segmenta creva tokom hirurške intervencije. Ovo je naročito značajno u slučaju postojanja angiodisplazije tankog creva, koja je standardnim metodama ispitivanja (ezofagogastroduodenoskopija, kolonoskopija) često nedostupna. Kombinovanu primenu selektivne arteriografije i metilenskog plavog prvi put su opisali 1978. godine Fogler i Golembe [3]. Prema dostupnim podacima iz literature prikazani bolesnik je prvi objavljeni slučaj na našoj teritoriji.

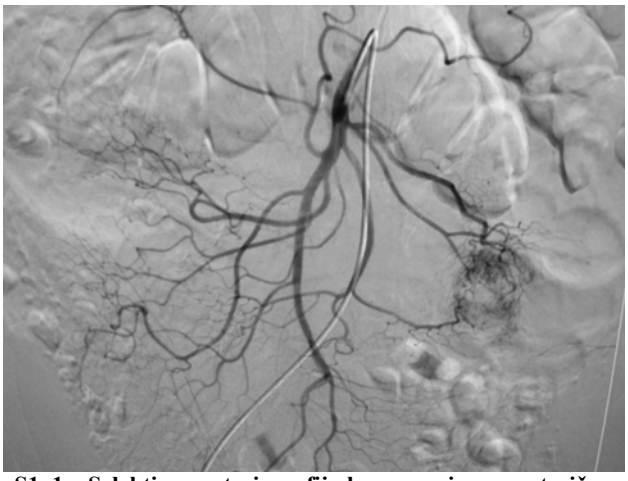
Prikaz bolesnika

Bolesnik, star 65 godina, hospitalizovan je kao hitan slučaj zbog masivnih hematohezija. Na prijemu vrednosti krvnog pritiska bile su 80/50 mmHg, puls 120/min, hematokrit 21%, hemoglobin 76 g/L i broj eritrocita $2,6 \times 10^{12}$/L. Hitno je izvršena nadoknada tečnosti i elektrolita, uz dodatak tri jedinice krvi i jedne jedinice sveže plazme. Nakon toga došlo je do poboljšanja opšteg stanja sa normalizacijom vitalnih parametara. U anamnezi bolesnik je naveo podatak da je krvarenje započelo pre dve godine u vidu blago sukrvičavih stolica i da se to povremeno javljalo. Tretiran je antihemoroidalnim mastima, zbog sumnje na postojanje unutrašnjih hemoroida, u trajanju od godinu dana. Tegobe su se neznatno smanjile. Ovo je bio prvi put da je zbog krvarenja primljen u bolnicu. Kliničkim i ultrazvučnim pregledom trbuha nije utvrđeno postojanje bilo kakvih abnormalnosti. Rektalnim tušeom dobijen je otisak stare koagulisane krvi, ali bez postojanja palpabilnih tumefakata. Indikovana je proksimalna endoskopija, kada je verifikovano postojanje blagog gastritisa, bez znakova svežeg krvarenja.

Nakon toga urađena je totalna kolonoskopija, uz otežanu interpretaciju zbog zaostalog sadržaja stare krvi u lumenu debelog creva. Pregled je ponovljen nakon dva dana, kada je konstatovan potpuno normalan nalaz. Tokom dijagnostičkih ispitivanja došlo je do ponovnog krvarenja sa blagim padom vitalnih parametara. Indikovana je digitalna suptrakciona selektivna arteriografija, kroz gornju i donju mezenteričnu arteriju. Desnim femoralnim pristupom uveden je kateter od 5 F (Pro Great, Terumo, Belgium) u izvorište gornje mezenterične arterije i ubrizgano je jodno, nejonsko, kontrasno sredstvo.

Utvrđeno je postojanje hipervaskularne zone sa intenzivnijim prebojavanjem u nivou jejunalnih grana gornje mezenterične arterije, koje je perzistiralo duboko kroz vensku fazu i odgovaralo angiodisplaziji.

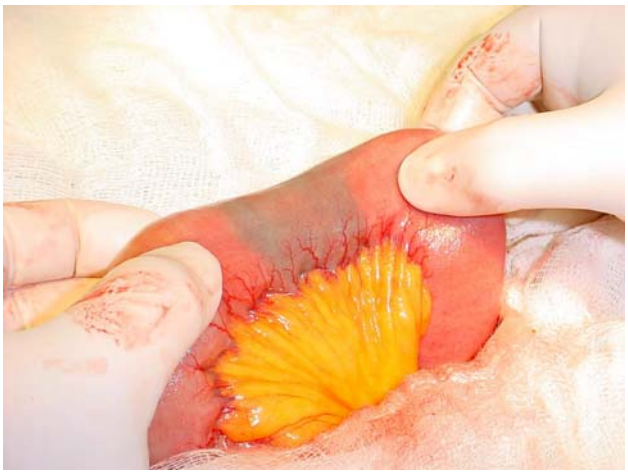
Kroz koaksijalno plasiran mikrokateter u arterijski fider angiodisplazije ubrizgano je 0,5 mL 1% sterilnog rastvora metilenskog plavog radi bojenja segmenta jejunuma i precizniju identifikaciju mesta kravrenja (slika 1).



S1. 1 – Selektivna arteriografija kroz gornju mezenteričnu arteriju sa nakupljanjem kontrasta u nivou jejunalnih grana

Nakon jednog sata od završetka selektivne arteriografije i aplikacije metilenskog plavog izvedena je hirurška intervencija.

Bolesnik je operisan u opštoj anesteziji. Trbuh je otvoren gornjom i srednjom medijalnom laparotomijom uz detaljnu eksploraciju trbušne duplje i retroperitonealnog prostora. Vrlo brzo identifikovan je segment jejunuma obojen plavim, na 20 cm od Treitzovog ligamenta (slika 2).



Sl. 2 – Prebojeni segment jejunuma nakon aplikacije metilenskog plavog

Urađena je resekcija vijuge jejunuma u dužini od 10-ak centimetara sa terminoterminalnom anastomozom. Na definitivnom histopatološkom preparatu, bojenom hematoksilin eozinom, PAS i trihromnim bojenjem, utvrđeno je postojanje angiodisplazije jejunuma. Neposredni postoperativni tok protekao je sasvim uredno, sa prestankom krvarenja. Bolesnik je kontrolisan svaka tri meseca, do dve godine nakon hirurške intervencije, sa potpuno urednim kliničkim, odnosno labaratorijskim nalazom i bez znakova aktivnog krvarenja.

Diskusija

Angiodisplazija tankog creva predstavlja stečeno, ređe urođeno, vaskularno oboljenje, izazvano hroničnom intermitentnom opstrukcijom mukoznih i submukoznih vena. Prvi put opisali su je 1960. godine Margolis i sar. [4]. Odgovorna je za 6% slučajeva krvarenja iz donjih partija digestivnog sistema.

Javlja se uglavnom kod starijih osoba, preko 60 godina života i posledica je nespecifičnih degenerativnih promena na malim krvnim sudovima, u zidu tankog creva. Česte muskularne kontrakcije prouzrokuju opstrukciju venskog sistema i povećanje intraluminalnog pritiska, sa konsekutivnom dilatacijom venula, lokalizovanim u zidu creva (najčešće submukozi). Progresijom promena zahvataju se prekapilarni sfinkteri, koji postaju nekompetentni, tako da se formiraju male arteriovenske komunikacije [5]. Najveći broj angiodisplazija protiče asimptomatski. Kliničke manifestacije vezane su za pojavu krvarenja, koje može biti akutno i hronično. Kod 85% slučajeva svih akutnih krvarenja uzrokovanih angiodisplazijom tankog i debelog creva dolazi do njihovog prestanka tokom konzervativnih mera lečenja. Kod preostaih 15% indikovana je hitna hirurška intervencija [6]. Najznačajniji problem u hirurškom lečenju predstavlja identifikacija mesta krvarenja, naročito u području tankog creva ili u tzv. „skrivenim" regijama koja se klasičnim endoskopskim procedurama kao što su ezofagogastroduodenoskopija i kolonoskopija, često nedostupna [7]. Savremeni dijagnostički postupci podrazumevaju primenu: radioizotopskog skeniranja uz pomoć ^{99}tehnecium obeleženih eritrocita, MSCT-a, NMR angiografije, endoskopske kapsule, koje sa određenim stepenom preciznosti mogu identifikovati mesto krvarenja u tzv. „skrivenim" regijama. Međutim, nedovoljno kliničko iskustvo sa jedne strane, kao i visoka cena aparata sa druge, limitiraju širu primenu ovih dijagnostičkih procedura. Kombinovana primena selektivne arteriografije i metilenskog plavog predstavlja lucidnu metodu identifikacije mesta krvarenja iz donjih partija gastrointestinalnog trakta čime se olakšava i ubrzava izvođenje hirurške intervencije. U odnosu na vreme izvođenja hirurške intervencije, primena metilenskog plavog može biti preoperativna i intraoperativna. Preoperativna primena podrazumeva aplikaciju metilenskog plavog odmah nakon izvođenja selektivne arteriografije.

Neposredno nakon toga izvodi se hirurška intervencija, najbolje tokom jednog sata. Na ovaj način prevenira se nepotrebno „razlivanje" boje unutar sistema krvnih sudova i time obeležavanje znatno šireg segmenta tankog creva sa nepotrebnim opsežnim resekcijama i opasnosti od malapsorpcionog sindroma [8, 9]. Intraoperativna primena podrazumeva da se tokom hirurške intervencije kroz preoperativno postavljeni angiografski kateter ubacuje metilensko plavo čime se prebojava segment tankog creva sa mestom krvarenja [10–12]. Ovu metodu moguće je primeniti i kod novorođene dece bez opasnosti od neželjenih reakcija [13].

Postojanje različitih metoda aplikacije u literaturi je posledica pre svega identifikacije i tretmana različitih uzroka krvarenja iz tankog creva sa jedne strane, kao i nedostatka iskustva, odnosno rezultata serija sa većim brojem bolesnika, sa druge. Ne postoje komparativne studije koje bi dale odgovor na pitanje o tome koja je od ove dve metode bolja i korisnija u kliničkoj praksi.

Skromno iskustvo autora ovog rada pokazuje da je preoperativna primena metilenskog plavog efikasnija i bezbednija, a da su tokom intraoperativne primene moguće tehničke poteškoće prilikom aplikacije metilenskog plavog, kao i rizik otežanog sprovođenja osnovnih principa asepse.

Zaključak

Kombinovana primena selektivne arteriografije i metilenskog plavog vrlo je korisna metoda u identifikaciji angiodisplazije tankog creva, čime se omogućava brza, jednostavna i poštedna hirurška intervencija, odnosno smanjuje se mogućnost opsežnih resekcija i malapsorpcionog sindroma. Neophodan uslov pravovremene dijagnoze je dobra saradnja radiologa i hirurga u izvođenju angiografije sa aplikacijom metilenskog plavog i, neposredno nakon toga, hirurške intervencije.

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I S T O R I J A M E D I C I N E

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Vojni sanitet tokom operacija Srpske vojske u severnoj Albaniji 1912–1913. godine

Military Medical Corps during operations of Serbian Army in northern Albania 1912–1913

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Uvod

Da li je već tokom izrade ratnog plana za rat sa Turskom Đeneralštab srpske vojske razmišljao o nastupanju u dva pravca: na jug Vardarskom dolinom i na zapad prema obali Jadrana preko severne Albanije do Medove i Drača? Da li je zbog toga njeno desno krilo bilo neuobičajeno jako ili je odluka o nastupanju na Jadran doneta kasnije?* Ne želeći da ulazimo u raspravu oko ovoga, moramo naglasiti da se Srbija posle Berlinskog kongresa 1878. našla u geopolitički veoma nesigurnom stanju, jer je njegovim odlukama bila lišena sigurnog prilaza morskim lukama kako na Egejskom, tako i na Jadranskom moru i ostavljena na milost Austrougarske njenim vojnim prisustvom u Novopazarskom sandžaku, odnosno Turske koja je ostala u posedu Vardarske doline do Soluna. Ovo stanje postalo je neodrživo u prvoj deceniji XX veka zaoštravanjem odnosa sa Austrougarskom, usled njene aneksije Bosne i Hercegovine i „Carinskog rata" kojim je pokušala da ekonomski uništi Srbiju, i usled dolaska mladoturskog režima na vlast u Carigradu sa njegovim neootomanskim ambicijama i svime što su one u sebi nosile [1–4].

Ratnim uspehom u Makedoniji posle Kumanovske i Bitoljske bitke glavni deo ratnog plana bio je ispunjen, ali ne i osiguran, zbog nastalih suprotnih aspiracija dve ratne saveznice, Srbije i Bugarske, u Makedoniji. Takođe, dve saveznice Srbije, Grčka i Crna Gora, nisu još završile svoje operacije − prva je u želji da ovlada Epirom bezuspešno opsedala Janjinu, a druga Lješ i Skadar. Osim toga, na teritoriji severne Albanije nalazile su se dosta snažne turske trupe u jačini od oko tri i po divizije regularne vojske i više bataljona sastavljenih od domorodačkog stanovništva, Albanaca i Muslimana, ugrožavajući time sigurnost zauzetog zaleđa u oblastima naseljenim buntovnim albanskim stanovništvom.

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* U pogledu Ratnog plana Srbije za rat sa Turskom postoje različita mišljenja po pitanju planiranja prodora srpske vojske na Jadran. Jedno, starije, ali vrlo relevantno i izneto bez ikakve rezerve, je mišljenje savremenika dr Stanoja Stanojevića, istoričara, profesora Univerziteta i člana Kraljevske srpske akademije nauka u njegovoj opširnoj monografiji o srpskoturskom ratu na str. 154 [2]: „... Pri izradi plana za rat protiv Turske srpski je Đeneralštab predvideo i izlaz na more, na Lješ i San Đovani di Medua, i na Drač. Zbog toga je đeneral Putnik i odredio da Treća armija bude znatno veća, no što je za to, neposredni njen zadatak – osvojenje Kosova i Metohije, bilo potrebno...." Drugo, novije, koje iznosi dr Savo Skoko [8] u svojoj monografiji o vojvodi Putniku (str. 314–15): „Albanska operacija nije bila predviđena srpskim ratnim planom, iako je izlazak na more bio jedan od ciljeva srpske državne politike toga doba. Verovatno se računalo da će crnogorska vojska brzo i lako zauzeti severnu Albaniju i Skadar.....Kada se pokazalo da su ta očekivanja nerealna... predsednik srpske vlade Nikola Pašić 2. novembra telegrafisao je Putniku da je opšta situacija vrlo povoljna i da bi bilo dobro da Srpska vojska izbije na Jadransko more... "

Skoko, pored ovoga, navodi da su postojale četiri varijante ratnog plana.

Milutin Lazarević, đeneralštabni oficir, u svojoj prvoj knjizi trilogije o srpskoturskom ratu [3] navodi dva podatka koji se odnose na planove vojvode Putnika:

1. (str.51): „...vojvoda Putnik nije ostavio za sobom nijedan dokumenat u kome bi bila izražena celina njegove zamisli..."; 2. (str.59): vojvoda u direktivi komandantu III armije kaže „...kad budete zauzeli Prištinu..osim Moravske divizije 2. poziva koja za prvi mah ostaje u Prištini, sve ostale vaše trupe imaju, bez zadržavanja, da produže nastupanje ka Skoplju."

Ni Živko Pavlović, najbliži saradnik vojvode Putnika ne potvrđuje postojanje planova za marš ka Jadranu. Na str. 91 [5] on kaže: "...Upućivanje naših trupa kroz severnu Arbaniju na Jadransko Primorje imalo je prvenstveno politički čin, a potom i vojnički. Kada se s naše strane osetilo da ubrzo mogu otpočeti pregovori za zaključenje primirja..tada se težilo da mi na Jadransko more izbijemo pre no što bi Velike Sile otpočele pregovore i posredovanja za mir, kako bi se one u momentu pregovora za mir stavile pred svršeni čin... koji bi nam mogao obezbediti davno željeno naše izbijanje na Jadransko more, čime bi se postigla ekonomska nezavisnost Srbije".

Bilo kako bilo, sve je izvedeno na zahtev Vlade u okviru njenih strateških opredeljenja, koja su se na kraju pokazala uzaludna.

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Stoga, i sa političke i vojne tačke gledišta činilo se opravdanim da se pristupi ostvarenju poduhvata kojim bi se osigurao izlaz na more i, smatralo se, stavile Velike sile pred svršeni čin, a istovremeno likvidirale turske trupe i time pomoglo Crnoj Gori u zoni Skadra koji su njene trupe uzaludno pokušavale da osvoje [5–8].

Izlazak na more

Trupe III armije, napredujući ka jugu, zaposedale su Kosovo, Drinska divizija 2. poziva 22. oktobra zauzela je Đakovicu, a Šumadijska divizija 1. poziva, 23. oktobra, Prizren. Kada su trupe III armije stigle u Prizren počeo je padati sneg na već kišama raskaljano zemljište, a uskoro je nastupila i jaka hladnoća i mraz.

Ipak, na zahtev srpske Vlade, Vrhovna komanda je odmah, 23. oktobra, izdala naređenje za pokret delova ovih divizija ka Jadranskoj obali u cilju zauzimanja luka u Medovi i Draču. U velikoj žurbi trupe su morale za nekoliko dana da se pripreme za pokret i reorganizuju za kretanje planinskim terenom, iako im je za taj poduhvat nedostajalo svega, počev od zimske opreme, pre svega odeće i obuće, u čemu su i inače oskudevale (jedinice Drinske 2. bile su u svojim narodnim odelima i opancima, već propalim tokom prethodnih ratnih operacija), preko namirnica (na jednog vojnika obezbeđeno je samo po dva hleba, uz nešto suve hrane za ceo put od planiranih pet dana) [5, 6, 8].

Nedostajali su potrebni lekovi, a sanitet je krenuo bez svoje transportne jedinice (sanitetska kolona), zbog neprohodnosti pravaca kojima je trebalo da se kreću trupe [5].

Trupe su podeljene u dve kolone koje će se posebno kretati sa sastajalištem u zoni Lješ – Medova [5]. Desna kolona (delovi Drinske 2: 7 pešadijskih bataljona V i VI puka, konjički divizion, dve brdske baterije topova, pioniri, pekari, deo bolničke čete) krenula je 28. oktobra iz Đakovice, da preko Spasa i Puki za pet dana stigne do Lješa.

U ovoj koloni u sastavu saniteta nalazili su se sledeći lekari [9–13]: referent saniteta divizije, potpukovnik dr Jordan Stajić; sanitetska četa: komandir major dr Jakov Brik, rez. kapetan 1. kl. dr Andra Jovanović (do 3.1.1913.), rez. kapetani 2. kl. dr Staniša Simić i dr Milivoje Ranković; V puk: rez. kapetan 2 kl. dr Svetislav Stefanović; VI. puk: rez. poručnik dr Mihailo N. Petrović (svi su oni kasnije radili u privremenoj bolnici u Lješu.) Ukupna jačina odreda iznosila je: 9 219 vojnika, podoficira i oficira.

Dok je napredovao, odred je imao nekoliko sukoba sa lokalnim Albancima, od kojih se jedan svršio stradanjem grupe od 100 vojnika koji su osiguravali relejnu službu. Drugi sukob odigrao se pri prelazu reke Drima, a treći po pristizanju na morsku obalu, gde je prethodnica od dva bataljona pešadije, jednog eskadrona konjice i dva topa morala da vodi borbu sa turskim trupama iz 53. redifskog (rezervnog) puka Debarske divizije koje su se zabarikadirale u lješkoj tvrđavi. Tom prilikom zarobljeno je 1 000 turskih vojnika, 19 oficira i dosta ratnog i sanitetskog materijala. Zauzećem Lješa uspostavljena je veza sa crnogorskim trupama, a potom je u Medovu upućen jedan bataljon pešadije da zajedno sa Crnogorcima čuva pristanište [4, 5, 8].

Sa pozadinom u novooslobođenim oblastima održavana je stalna veza putem uspostavljene relejne linije čije su stanice bile smeštene u pojedinim naseljenim mestima duž puta [5].

Ovaj borbeni marš završen je za devet dana, uz dosta obolelih vojnika. Vojnici, već iscrpljeni prethodnim ratnim operacijama, a sada izloženi teškim vremenskim prilikama, loše odeveni i obučeni, gladni i promrzli, jer su morali noću da spavaju pod otvorenim nebom i bez šatorskih krila, masovno su se razbolevali. Kako nije postojala transportna sanitetska služba, bolesni vojnici ostavljani su u usputnim albanskim selima gde su se nalazile relejne stanice, kod Albanaca koji su za to novčano nagrađeni [5].

Posle zauzimanja Lješa obrazovana je vojna bolnica, čiju je upravu preuzeo major dr Jakov Brik. U njoj, sve do kraja boravka srpskih trupa na albanskom primorju, lečeni su svi srpski vojnici koji su se nalazili u severnoj zoni. Bolovanja je bilo dosta, najviše od malarije, trbušnog tifusa i dizenterije, pa je i umiranje bilo veliko, ponekad i do 15 vojnika dnevno.

Leva kolona (trupe iz sastava Šumadijske divizije 1. poziva: šest bataljona iz sastava X, XI, XII i XIX puka podeljenih u dva kombinovana puka, prvi i drugi, jedan konjički eskadron, jedna brdska baterija, dve mitraljeske čete, pioniri i deo bolničarske čete, ukupno oko 6 500 vojnika, podoficira i oficira) krenula je iz Prizrena 27. oktobra pravcem Vezirov most - Fani - Oroši - Lješ, putem kojim se, čak, ni turske vlasti nisu usuđivale da prođu, jer su tu živela albanska plemena koja su se nekoliko meseci ranije pobunila protiv njih i zauzela Kačanik i Skoplje. Njih je, usput, trebalo razoružati, kako bi se obezbedila bezbedna komunikacija od Prizrena do mora.

U sastavu saniteta, koji se sastojao iz dela sanitetske čete bez transportnih sredstava i bez dovoljno opreme i lekova, bili su [9–13]: referent saniteta odreda major dr Petar Popović (od januara 1913, rez. kapetan 2 kl. dr Sima Petrović); lekari odreda: akt. poručnici dr Vladimir Stanojević (2. puk) i dr Dušan Kopša (1 puk); sanitetska četa: rez. kapetan 2 kl. dr Milan Simić-Popović, poručnik dr Đorđe Siber,vojni obveznik dr Stevan Ivanić i vojni obveznik na odsluženju vojnog roka dr Milorad J. Bošković.

Odred je imao sreću da ne dođe u sukob sa lokalnim stanovništvom koje su u početku činili muslimanski, a zatim katolički Albanci, ali je zato pretrpeo gubitke usled veoma lošeg vremena, maršovanja po vrletnom bespuću i poplavljenom terenu; obolelo je skoro 25% ljudskog sastava. Posle 10-dnevnog putovanja odred je stigao do Lješa, gde je zatekao ljudstvo Drinske 2 i sa njime učestvovao (jedna četa i jedan brdski top) u borbi za osvajanje tvrđave [5].

Dana 9. novembra odred je krenuo na put duž morske obale 60–70 km preko reke Maće do Drača, gde je stigao 16. novembra oduševljeno dočekan od hrišćanskog stanovništva. Usput je odred osvojio Kroju (12.11) i Tiranu (15.11), dok su se delovi razbijene turske 21. nizamske divizije (divizija aktivne vojske) povukle na jug, u Kavaju [5].

U Draču sanitet je odmah obrazovao ambulantu i bolnicu, koje će, kako se pokazalo, biti veoma zaposlene tokom celog boravka. Njihovu upravu preuzeo je do bolesti dr Dušan Kopša [11, 12].

Iz Tirane odred je uspostavio vezu preko Kavadara i Kraba sa Elbasanom, gde su se od 15. novembra nalazili delovi Moravske divizije 2. poziva, pošto su prethodno zauzeli Ohrid i Debar. Po primirju zamenio ih je pojačani XVIII puk Dunavske divizije 1. poziva (lekari: kapetan 1 kl. dr Ljubiša Vulović i kapetan 2 kl. dr Kosta Jovanović, u zavojištu kapetan 2 kl. dr Dragutin Radišić), a od januara 1913. Moravska brigada, koja je od 11. do 29. marta imala više sukoba sa ostacima Džavid-pašine Vardarske armije, potisnuvši ih ka Fijeri. U Elbasanu i okolini ona je ostala do 22. aprila, kada se povukla na novu (privremenu) državnu granicu [5, 11, 12].

U sanitetu brigade bili su [11, 12]: 1. prekobrojni puk – rez. kapetan 2 kl. dr Spira Nikolić i poručnik dr Milosav Petrović; 2. prekobrojni puk (deo?) – rez. kapetan 2 kl. dr Borivoje Beraha; 1. poljska bolnica – (od marta) poručnik dr Dobrivoje Stojnić; 2. poljska bolnica – rez. poručnik dr Dimitrije Kalijadis.

Ove dve bolnice radiće u Elbasanu, a dr Stojnić će kraće vreme biti detašovan u Ćukus, gde je otvorio etapnu bolnicu.

Na taj način završeno je posedanje Albanije do linije Drač – Elbasan – Struga i obrazovana dva administrativna okruga: Lješki i Drački. Južno od ove linije albanska plemena obrazovala su svoju privremenu vladu u Valoni.

Snabdevanje srpskih trupa na albanskom primorju sporazumno je preuzela Grčka svojim brodovima. Kasnije je korišćena i veza sa severnim jadranskim lukama (Trst), kada je turska gusarska krstarica „Hamidije“ počela sa svojim prepadima na brodove u južnom Jadranu. Kada je ta linija uspostavljena, popravljeno je i snabdevanje trupa naminicama i drugim potrepštinama [5].

Dana 20. novembra Srbija, Crna Gora i Bugarska potpisale su primirje sa Turskom (Grčka je odbila, zbog Janjine oko koje je još ratovala), koje je sutradan stupilo na snagu.

Dana 25. novembra objedinjene su srpske trupe u „komandu primorskih trupa“ sa sedištem prvo u Draču, potom u Lješu.

Kakvi su problemi u sanitetu ove vojne ekspedicije bili, svedoči nam dr Sima Petrović: „....iz Skoplja poslat u Albaniju, gde (sam) vršio dužnost referenta saniteta Albanskog odreda u Draču pored dužnosti upravnika dračkih bolnica..... Po oboljenju trupnog lekara dr D. Kopše svakodnevno obilazio i logor na Škumbi. Kada su se svi naši lekari u Draču razboleli (tifus i pegavi tifus) vraćen u Drač, gde (sam) ostao skoro sam i sve: vršilac dužnosti referenta Albanskog odreda, upravnik i lekar svih 12 dračkih bolnica sa preko hiljadu obolelih i ranjenih, lečeći i obolele kolege, dr D. Kopšu, dr M. Simića i dr Đ. Sibera, dok nisam i sam teško oboleo od pegavog tifusa i 10. aprila 1913. lađom prenet i ostavljen na bolovanju u Krfu...“ [9].

To potvrđuju i podaci iz personalnog kartona jednoga od njih, dr Dušana Kopše, koji je 1913. godine u Albaniji preboleo dizenteriju, trbušni tifus i pleuropneumoniju i zbog toga bio pet meseci na bolovanju [11, 12].

Cela ova ekspedicija bila je sa sanitetskog gledišta vrlo komplikovana, izvedena po zimskom vremenu, bez trupa pripremljenih za takav poduhvat, bez dovoljno namirnica i lekova, bez podnošljivih smeštajnih uslova i bez mogućnosti da se sprovedu prave preventivne mere u cilju zaštite od zaraznih bolesti. Rezultat svega toga je veliki procenat obolelih i tokom samoga marša do cilja, a zatim i obolevanja od raznih zaraznih bolesti (malarija, dizenterija, pegavi tifus, trbušni tifus, rekurens) od čega su oboljevali i sami lekari, kako se vidi iz zapisa dr Sime Petrovića i kartona dr Dušana Kopše. Nisu se bolje provele ni kasnije prispele trupe, koje su, transportovane brodovima, stigle na primorje pri kraju zime i početkom proleća, jer se uslovi boravka nisu mogli promeniti.

Primorske trupe

Posedanjem zone Lješ – Medova bilo je završeno strateško opkoljavanje Skadra u širem obimu koji crnogorske trupe nisu mogle same da zatvore.

Iako je primirje bilo na snazi, povremeno je kršeno, pa je u jednom takvom sukobu kod sela Dajča tokom 17. i 18. novembra došlo do stradanja veće grupe srpskih vojnika, izgubljeno je 320 vojnika i major Borko Paštrović [5].

Zimsko vlažno vreme, močvarno zemljište, loši smeštajni uslovi i ishrana doprineli su pojavi epidemije dizenterije i malarije tako da je samo u bolnici u Lješu do kraja januara umrlo 378 srpskih vojnika [5].

Primirje je isteklo 20. januara 1913.

U obnovljenim sukobima od 25. januara, dok su Crnogorci napadali i ginuli na Bardanjoltu, trupe Drinske 2. osvojile su Bušatske položaje uz neznatne gubitke. U noći 26–27. januara oni su nastavili neuspešne napade na teško utvrđenu Brdicu pretrpevši teške gubitke od preko 1 800 poginulih, ranjenih i nestalih. Na žalost, ovi podaci su neprecizni, kako to navodi Živko Pavlović u svojoj studiji. Samo V. puk i 4. bataljon XI puka izgubili su 861 vojnika, oficira i podoficira. Prema jednome turskom izveštaju, u toku ove noćne borbe, u kojoj je deo prodrlih srpskih vojnika bio opkoljen, zarobljeno je 632 srpska vojnika, podoficira i oficira, a na bojištu su nađena i 104 ranjenika [5, 6, 8].

Obrazovanje Primorskog kora srpske vojske

Ne mogavši da osvoji Skadar, kralj Nikola zatražio je jače angažovanje srpske vojske, te je srpska vrhovna komanda 8. februara obrazovala Primorski kor na čelu sa đeneralom Petrom Bojovićem. Za načelnika saniteta postavljen je pukovnik dr Đoka Vladisavljević. Njegov ađutant bio je dr Žarko Živković, lekar na odsluženju vojnog roka [5, 6, 8, 11, 12].

U sastav korpusa ušle su jedinice dosadašnjih Primorskih trupa i Drinska divizija 1. poziva, koju je trebalo iz Soluna prevesti brodovima do Medove. U stvarnosti je pred Skadar došao samo V i XVII puk sa delovima artiljerije, pionira i aeroplansko odeljenje sa četiri aviona, dok su ostale jedinice divizije ostale u Solunu, odakle su vraćene u Skoplje, jer su u međuvremenu velike sile izvršile snažan pritisak na grčku i srpsku vladu i zapretile blokadom Jadranske obale [5].

Sa ovim jedinicama došao je i deo saniteta divizije [9–15]: V puk – kapetani 2. kl. dr Jovan Milosavljević i (rezervni) dr

Dragoljub Milošević; XVII puk – rez. kapetan 2 kl. dr Vojislav St. Popović i poručnik dr Aleksandar Vesić; deo bolničarske čete – v.d. komandira rez. kapetan 1 kl. dr Momčilo Ivković i rez. poručnik dr Dušan Radaković.

Dr Radaković u Lješu radiće u srpskoj vojnoj bolnici, a dr Ivković istovremeno biti upravnik svih onde postojećih bolnica: srpska i dve ruske, kaufmanska i peterburška jelisavetinska, kojima će se od 18. aprila pridružiti i ruska bolnica "Gorod Moskva". Tom prilikom je ruskim bolničkim brodom "Peterburg" iz medovske luke evakulisano 800 obolelih srpskih vojnika, od toga 250 tifusara (pegavac), dok je na lečenju u ruskim bolnicama ostalo još njih 700 [16].

U Solunu je, kao rukovodilac ambulante za srpske vojnike koji su ukrcavani ili su vraćani ostao kapetan 1. kl. dr Platon Papakostopulos sa pomoćnim osobljem.

Ova dva novoprispela puka sa već u Albaniji prisutnim srpskim jedinicama u zoni Skadra i Drača činili su „Primorski kor" čija je dužnost bila da obezbeđuju zaposednutu severnu Albaniju i da pomognu crnogorskim trupama u osvajanju utvrđenog Skadra.

Bilo je predviđeno da kor broji 30 450 ljudi, 4 697 konja, 3 714 volova, 41 top sa 90 kara, 20 mitraljeza, četiri aeroplana i 1 905 kola. U stvarnosti je on brojao samo 17 000 ljudi, 40 topova raznih kalibara i namene, četiri aeroplana i 3 260 konja i volova, ostatak je zbog protivljenja velikih sila zadržan u Solunu [5].

Trupe su prebačene u tri konvoja [5]: 1. konvoj (četiri broda) sa dunavskim artiljerijskim divizionom (lekar dr Mil. Kojić ?) (u buri, jedan brod pretrpeo je brodolom udarom o stenu, ali su se svi, osim jednog vojnika, spasli); 2. konvoj (šest brodova) sa XVII pukom, avionima i hranom (za vreme iskrcavanja u medovskoj luci napadnut je od turske lake krstarice „Hamidije", kojom prilikom je poginulo 60 i ranjeno 20 vojnika); 3. konvoj (16 brodova) sa štabom kora, V pukom, artiljerijom, pionirima, inžinjerijom, pontonima, sanitetskim slagalištem, osobljem i opremom dve ruske bolnice, moskovskom i petrogradskom, koje su došle u pomoć Srpskoj vojsci i Crnogorcima. Ovaj konvoj, uprkos peripetija koje su stvarale Velike sile pritiskujući grčku vladu ipak je stigao do odredišta, praćen grčkim ratnim brodovima radi zaštite od „Hamidije".

Imajući u vidu veoma loše higijenske i vremenske prilike, zbog velikog procenta obolevanja prisutnih trupa, pri čemu su se pored sezonskih bolesti širile i ozbiljne zarazne bolesti (malarija, dizenterija, trbušni i pegavi tifus, rekurens) koje nisu mimoišle ni sanitetsko osoblje, sanitet je bio prinuđen da, pored glavnih bolničkih centara za obe grupacije (Drač i Lješ), otvara i stacionare u Tirani, Kavaji, Kroji i Elbasanu koje su opsluživali prisutni trupni lekari. Nisu sačuvani tačni podaci o broju lečenih i umrlih, ali se već iz navedenog izveštaja dr Sime Petrovića vidi koliko je broj obolelih bio lečen samo u jednoj, dračkoj bolnici. Uspostavljenim morskim saobraćajem između Soluna i albanskih pristaništa bilo je moguće da se teško oboleli transportuju do Soluna, uprkos ometanjima velikih sila.

Pored srpske, u Lješu su se nalazile i dve već pomenute ruske bolnice i vojišno sanitetsko slagalište, kao i baza hrane i furaži.

Po prispeću pojačanja izvršena je definitivna podela sektora, tako da je crnogorska vojska držala sektore Taraboša i Bardanjolta, a srpska sektor Brdica, sa kojih je trebalo izvršiti napade u cilju osvajanja Skadra, o čemu su napravljeni detaljni zadaci, a vrhovna komanda nad srpskim i crnogorskim trupama poverena đeneralu Petru Bojoviću.

Međutim, velike sile izvršile su veliki pritisak na srpsku vladu da povuče svoje trupe, preteći ne samo blokadom, već i bombardovanjem srpskih i crnogorskih položaja. Uz to, definitivno su donele odluku da Skadar pripadne budućoj državi Albaniji. Našavši se pred pretnjom rata sa Austrougarskom, koja je na granici Srbije koncentrisala oko 200 000 vojnika prema 25 000 srpskih, srpska vlada je 28. marta 1913. donela odluku o prekidu opsade Skadra i povlačenju svojih trupa iz severne Albanije u Solun[*]. Tako su se raspršila njena nadanja da će stavljanjem velikih sila pred svršeni čin obezbediti direktan morski prilaz.

Trupe su postepeno tokom aprila brodovima vraćene u Solun.

Crnogorci su pregovorima sa Esad-pašom uspeli da uđu u Skadar u kome je došlo do gladi, ali su pod pritiskom i pretnjama velikih sila bili prinuđeni da ga napuste.

Ova avantura srpske vojske, u kojoj je sanitet uzeo puno učešće pod vrlo nepovoljnim vremenskim, higijenskim i organizacionim okolnostima, a njegovi lekari sreli se sa pegavcem, nju je koštala gubitka od oko 3 500 vojnika poginulih i umrlih, a mnogi od njih su se vratili trajno oštećenog zdravlja.

I ovom prilikom kao i u kasnijim ratovima, srpski vojni sanitet je bespogovorno učestvovao tokom celoga trajanja ove višemesečne operacije, iako je u njega ušao potpuno nepripremljen ili, po rečima đenerala Živka Pavlovića [5], „sanitetska služba nije bila organizovana, lekova skoro nije ni bilo", on je požrtvovano pratio svoje jedinice i u teškim okolnostima pokušavao da se organizuje i da pruži sve što se u takvim okolnostima moglo, trpeći i sam te iste nepovoljne životne i sanitarne uslove i bolujući od istih bolesti kao i oni kojima je svoju pomoć pružao.

Zaključak

Sa ove velike (skoro 100 godina) vremenske distance i poučeni mnogim sledujućim primerima iz burnih događaja u kojima su naši prethodnici ili i mi sami učestvovali, ne možemo da se uzdržimo od zaključka da je sanitet najčešće bio samo pasivni učesnik događaja na čije medicinsko oblikovanje nije mogao da utiče i da su iz toga proizašle mnoge nepotrebne žrtve, kako među onima koji su bili briga saniteta, tako i u samome sanitetu. Što je najtragičnije, u svođenju istorijskih događanja, o radu vojnog saniteta teško da bi se našla i po koja

[*] Naročito su aktivne u insistiranju i pretnjama bile Italija i Austrougarska zbog svojih aspiracija prema Jadranskoj obali, uz snažnu podršku Nemačke. Sa druge strane, sile „Srdačnog sporazuma" (Francuska, Velika Britanija i Rusija) nisu se još osećale dovoljno spremnim da se upuste u dalje zaoštravanje situacije koja bi mogla eskalirati do opšteg sukoba. Tako se desilo da su se sve u tome trenutku zajedno oduprle srpskim i crnogorskim aspiracijama. Opšti rat zbog sukobljenih interesa bio je za kratko odložen [1, 3–5].

reč, iako je on po svojoj prirodi ne samo najplemenitiji deo vojne mašinerije, već i onaj od čijeg je rada njegovim mnogostrukim učinkom zavisila borbena gotovost vojne sile, počev od preventive (očuvanja zdravlja), preko blagovremeno i stručno ukazane pomoći i rehabilitacije ranjenih i obolelih, do njihovog uspešnog povratka u borbene redove.

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Позив на рекламирање у 2011. години

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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.

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Examples of references:

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