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S A D R Ž A J / C O N T E N T S

Ovogodišnji dobitnici Nobelove nagrade za fiziologiju ili medicinu (s leva na desno): Harald zur Hausen (Nemačka), Françoise Barré-Sinoussi (Francuska) i Luc Montagnier (Francuska).
H. zur Hausen je nagrađen za otkriće humanih papiloma virusa (HPV), uzročnika cervikalnog karcinoma, drugog najčešćeg karcinoma kod žena, a F. Barré-Sinoussi i L. Montagnier za otkriće virusa humane imunodeficijencije (HIV), uzročnika sindroma stečene imunodeficijencije (AIDS).

The winners of the Nobel Prize in Physiology or Medicine 2008 (from the left to the right): Harald zur Hausen (Germany), Françoise Barré-Sinoussi (France) and Luc Montagnier (France).
H. zur Hausen was awarded for finding human papilloma viruses (HPVs) that cause cervical cancer, the second most common cancer among women, and F. Barré-Sinoussi and L. Montagnier for their discovery of human immunodeficiency virus (HIV) which causes AIDS.

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

Nobelova nagrada za medicinu za 2008. godinu

The Nobel Prize in medicine 2008

Nada Kuljić-Kapulica

Vojnomedicinska akademija, Institut za mikrobiologiju, **Beograd**

Nobelova nagrada za medicinu za 2008. godinu pripala je istaknutim naučnicima na polju virusologije.

Polovina nagrade pripala je Haraldu zur Hausenu iz Hajdelberga, za istraživanje iz oblasti humanih papiloma virusa (HPV), dok su drugi deo nagrade ravnomerno podelili francuski naučnici Farnçoise Barré-Sinoussi i Luc Montagnieru koji su otkrili virus humane imunodeficijencije (HIV) i dali doprinos istraživanju ovog virusa.

Harald zur Hausen (rođen 1936), profesor u penziji i bivši rukovodilac i naučni direktor Nemačkog centra za istraživanje karcinoma u Hajdelbergu, među prvima ukazao je na moguću ulogu virusa u nastajanju tumora kod ljudi i istakao da se više od 10% svih slučajeva kancera povezuje sa perzistentnom virusnom infekcijom. On je dokazao da postoje onkogeni tipovi, humanog papiloma virusa (HPV) koji izazivaju cervikalni karcinom, po učestalosti drugi karcinom kod žena (500 000 obolelih godišnje). Dokazao je virusnu DNK u malignim ćelijama tumora.

Još 70-tih godina prošlog veka zur Hausen je postavio hipotezu o ulozi humanog papiloma virusa u cervikalnom kanceru. Svojim istraživanjima dokazao je da onkogeni virus integriše svoj DNK u genom ćelije domaćina. Ispitivanjem tumorskih ćelija dokazani su HPV geni koji omogućavaju ćelijsku proliferaciju (E6 i E7 geni). zur Hausen je otkrio da u famiji Papilomavirusa postoji više tipova, a da samo određeni tipovi imaju onkogeni potencijal. Dugogodišnjim istraživanjima otkrio je i istakao značaj onkogenih tipova HPV16 i HPV18 kod bolesnica sa cervikalnim kancerom i dokazao da se oni najčešće nalaze u ovom tumoru (kod 70% cervikalnih karcinoma u svetu).

Ova otkrića doprinela su razjašnjavanju patogeneze HPV infekcije, razumevanju mehanizama onkogeneze i ćelijske transformacije, kofaktora virusne perzistencije i najzad razvoju profilaktične vakcine protiv HPV infekcije. Danas je poznato preko 100 tipova HPV (40 se nalazi u infekcijama genitalnog trakta, a 15 onkogenih tipova u cervikalnom karcinomu, dok se pojedini tipovi HPV nalaze i u drugim tumorima ljudi).

Farnçoise Barré-Sinoussi (rođena 1947), profesor i direktor Jedinice za kontrolu retrovirusnih infekcija u Odeljenju za virusologiju Pasterovog instituta u Parizu i Luc Montagnier (rođen 1932), profesor u penziji i direktor Svetske fondacije za istraživanje i prevenciju AIDS u Parizu, otkrili su virus humane imunodeficijencije 1983. godine.

Kratko posle pojave nepoznate smrtonosne bolesti u svetu ovi naučnici uspeli su da identifikuju njenog uzročnika, što je nesumljivo bio ogroman uspeh nauke. Otkrili su virus iz limfne žlezde obolelog sa početnim stadijumom stečene imunodeficijencije i dokazali aktivnost enzima reverzne transkriptaze, kao znak prisustva replikacije retrovirusa. Takođe, dokazali su retrovirusne partikule u inficiranim ćelijama. Dokazano je da izolovani virus napada imunski sistem, odnosno inficira i ubija limfocite i reaguje sa antitelima kod inficiranih bolesnika. Razaranje imunskog sistema ima za posledicu nesposobnost odbrane organizma, čak i od uobičajenih mikroorganizama. Nasuprot tada poznatim humanim onkogenim retrovirusima ovaj novootkriveni virus nije dovodio do nekontrolisanog ćelijskog rasta.

Ubrzo posle otkrića virusa dokazano je da je on uzročnik sindroma stečenog gubitka imuniteta (AIDS), pa su dalja istraživanja išla u pravcu kloniranja HIV genoma i razjašnjavanju replikativnog ciklusa virusa i patogeneze bolesti, kao i interakcije virusa i domaćina.

Ova otkrića doprinela su razvoju metoda za dijagnostiku infekcije, kao i za skriniranje produkata krvi i predstavljale su osnov za uspešan antiretrovirusni tretman.

Kao rezultat ovih istraživanja razvijeno je nekoliko grupa antiretrovirusnih lekova koji su omogućili stavljanje bolesti pod kontrolu i poboljšanje kvaliteta života obolelih.

Ovo otkriće označilo je početak borbe protiv globalne, ubikvitarne epidemije koja je zahvatila oko 1% svetskog stanovništva i koja i dalje traje.

Otkrića ovogodišnjih dobitnika Nobelove nagrade za medicinu predstavljaju ogroman doprinos nauci i razvoju medicine, uopšte. Zahvaljujući njima, omogućeno je dalje rasvetljavanje zagonetke tumora ljudi i postepeno rešavanje globalnih bolesti kakav je AIDS.

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Позив на рекламирање у 2008. години

У прилици смо да вам понудимо могућност оглашавања и рекламирања производа и услуга у часопису „**Војносанитетски преглед**“ (ВСП). То је сигурно најбољи вид и најзаступљенији начин упознавања евентуалних корисника са вашим услугама и производима.

Часопис „**Војносанитетски преглед**“, званични орган лекара и фармацеута Војске Србије, научно-стручног је карактера и објављује радове из свих области биомедицине и фармације. Радове равноправно објављују стручњаци из војних и цивилних установа и из иностранства. Штампа се на српском и енглеском језику. Часопис излази већ 75 година, а непрекидно од 1944. године до сада. Једини је часопис у земљи који излази месечно (12 бројева), на око 100 страна А4 формата, а повремено се објављују и тематски додаци (суплементи). Путем размене или претплате ВСП се шаље у 23 земље света. Радове објављене у ВСП-у индексирају: *Science Citation Index Expended (SCIE)*, *Journal Citation Reports/Science Edition*, *Index Medicus (Medline*), *Excerpta Medica (EMBASE), EBSCO* (преко ове базе ВСП је *on line* доступан од 2002. године у *pdf* формату) и *Biomedicina Serbica*.

Цене реклама и огласа у часопису „Војносанитетски преглед“ у 2008. години су:

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4.	Оглас у боји А4 формата за целу годину (11-12 бројева)	250 000,00 динара
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8.	Оглас у боји на корицама К2 и К4 за целу годину (11-12 бројева)	400 000,00 динара

За сва објашњења, упутства и понуде заинтересовани контактирају редакцију часописа „Војносанитетски преглед“. Средства се уплаћују на жиро рачун код Управе јавних плаћања у Београду број: 840-941621-02 **ВМА (за Војносанитетски преглед или за ВСП)**, **ПИБ 102116082**. Уплатницу (доказ о уплати) доставити лично или поштом (писмом, факсом, *e-mail*-ом) на адресу: Војносанитетски преглед, Црнотравска 17, 11000 Београд; тел/факс: 011 2669 689, e-mail: vmaini1@eunet.yu или vmaini2@eunet.yu

O R I G I N A L N I Č L A N C I UDC: 616.314.17-008.1:616.379-008.64(497.11)

Stanje tkiva periodoncijuma kod bolesnika sa dijabetesom melitusom u populaciji Beograda

Periodontal condition in diabetics in Belgrade

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Apstrakt

Uvod/Cilj. *Diabetes mellitus* (DM) kao oboljenje u čijoj osnovi jeste metabolički poremećaj odražava se na funkcionisanje brojnih organa. Stoga, česte manifestacije dijabetesa predstavljaju i gingivitis i parodontopatija. Cilj rada bio je da se ispita uticaj različitih faktora (pol, starost, trajanje DM, higijena usta i zuba) na oralne manifestacije dijabetesa. **Metode.** Ispitana je grupa bolesnika obolelih od DM i kontrolna grupa zdravih osoba. Grupu ispitanika činila su 52 odrasla bolesnika, starosti 18–79 godina, oba pola (33 žene i 19 muškaraca), obolela od dijabetesa melitusa tip 1 (35 ispitanika) i tip 2 (17 ispitanika), lečena u Institutu za endokrinologiju, dijabetes i bolesti metabolizma Kliničkog centra Srbije. Srednja starost ispitanika bila je 54,6 godina, a srednje trajanje dijabetesa 11,1 godina. Kontrolna grupa formirana je od 67 dobrovoljaca iz Beograda, studenata Stomatološkog fakulteta Univerziteta u Beogradu, starosti 19–24 godine, oba pola (47 ženskih i 20 muških), bez parodontopatije i sistemskih bolesti. Status periodoncijuma bolesnika definisan je na osnovu merenja indeksa plaka, gingivnog indeksa i dubine periodontnih džepova. **Rezultati.** Dobijeni rezultati ukazuju na povećanu učestalost i težinu parodontopatije kod bolesnika sa dijabetesom. Utvrđeno je da značajan negativni uticaj na stanje preostalih zuba kod bolesnika sa dijabetesom imaju dužina trajanja bolesti, starost bolesnika i loša oralna higijena, dok je pol bez uticaja na posmatrane promene. **Zaključak.** Pokazatelji nivoa oralne higijene i stanje periodontnog tkiva (indeks dentalnog plaka, gingivni indeks, dubina periodontnih džepova) značajno su pogoršani kod dijabetesnih bolesnika. Negativan uticaj na broj preostalih zuba imaju starost (> 50 godina), dužina trajanja dijabetesa (> 20 godina) i loša oralna higijena. Polovinu ispitanih bolesnika zbog gubitka svih ili velikog broja zuba nosi parcijalnu ili totalnu protezu, što pokazuje da je parodontopatija značajna komplikacija DM.

Ključne reči:
dijabetes melitus, tip 1; dijabetes melitus, tip 2; periodontalne bolesti; srbija; faktori rizika.

Abstract

Background/Aim. Diabetes mellitus (DM) as a complex metabolic disease influences functioning of numerous organs. Therefore, frequent diabetic complication is chronic periodontitis. The aim of the study was to investigate the influence of various risk factors, like age, sex, duration of DM, oral hygiene, on oral manifestations of diabetes. **Methods.** The group of diabetics included 52 adult patients, 18–79-year of age, both sexes (33 females, 19 males) out of which 35 patients were with diabetes mellitus type I and 17 with type II. Mean age of the patients was 54.6 years and the mean duration of diabetes was 11.1 years. The controls consited of 67 volunteers from the city of Belgrade, 19–24-year of age, both sexes (47 females, 20 males) with no paradonthopathy and non-systemic diseases. Estimation of periodontal status of the patients was performed by measuring dental plaque index, gingival index and periodontal pockets' depth. **Results.** The results suggest an increased incidence and severity of periodontitis in diabetic patients. It was established that duration of diabetes, patients' age and bad oral hygiene had a negative influence on status of remained teeth in diabetics, while the sex had no influence on parameters monitored. **Conclusion.** Indices of oral hygiene level and periodontal status (dental plaque index, gingival index and periodontal pockets' depth) were significantly worsened in patients with diabetes. Negative influence on remained teeth had patients' age (>50 years), duration of diabetes (> 20 years) and bad oral hygiene. About half of the patients had a total or partial bridge suggesting that periodontitis is significant complication of diabetes mellitus.

Key words:
diabetes mellitus, type 1; diabetes mellitus, type 2; periodontal diseases; yugoslavia; risk factors.

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Uvod

Diabetes mellitus (DM) jeste oboljenje u čijoj osnovi postoji metabolički poremećaj koji se karakteriše hroničnom hiperglikemijom nastalom zbog apsolutnog ili relativnog nedostatka insulina. Pored poremećaja u metabolizmu glukoze, poremećen je i metabolizam lipida i proteina. Tokom trajanja dijabetesa, uz hroničnu hiperglikemiju, dolazi do patoloških promena na raznim organima (oko, bubrezi, nervi, srce, krvni sudovi) [1].

Dijabetes je po zastupljenosti jedno od vodećih hroničnih oboljenja, kako u našoj zemlji, tako i u svetu. Ovo oboljenje pokazuje postepen porast incidencije (naročito DM tip 2), pa se može govoriti i o pandemiji ove bolesti. Procenjuje se da je broj obolelih u Srbiji 200 000–250 000, tj. zastupljenost je oko 2,5%, što je kao u zemljama u okruženju [1]. Pretpostavlja se da na svakog registrovanog bolesnika dolazi još jedan nedijagnostikovani slučaj. Podaci za Beograd pokazuju da incidencija dijabetesa iznosi oko 0,24%, a prevalencija 2,6% [2].

Zbog ogromne učestalosti, parodontopatija, takođe, predstavlja ozbiljan medicinski, ekonomski, pa i socijalni problem. Kod osoba obolelih od dijabetesa melitusa nastaju teška oštećenja periodoncijuma, obilno gnojenje iz periodontnih džepova, resorpcija vilične kosti i ispadanje zuba, a česta je i pojava akutnih periodontnih apscesa zbog oštećenja krvnih sudova i pada imunobiološkog potencijala [3].

Opisane su brojne promene u usnoj duplji dijabetesnih bolesnika, kao što su suvoća sluzokože, osećaj pečenja usta i jezika, smanjen protok salive, promene oralne flore sa predominacijom *Candide albicans*, hemolitičkih streptokoka i stafilokoka, učestala pojava karijesa. Ove promene, naročito, zapažene su kod loše kontrolisanog dijabetesa [4, 5].

Iako definitivni zaključci o specifičnim efektima dijabetesa na periodoncijum još uvek nisu doneti, opisane su različite promene kod ovih bolesnika, kao što su tendencija ka hiperplaziji gingive, polipi gingive, polipoidne proliferacije gingive, formiranje apscesa, zapaljenje periodoncijuma i gubitak zuba. Najozbiljnija promena kod loše kontrolisanog dijabetesa melitusa jeste slabljenje odbrambenih mehanizama i povećana sklonost ka infekciji, koja vodi destruktivnoj parodontopatiji [4, 5].

Cilj rada bio je da se ispita uticaj starosti, pola, dužine trajanja dijabetesa i oralne higijene na stanje tkiva periodoncijuma dijabetesnih bolesnika.

Metode

Grupu ispitanika činila su 52 odrasla bolesnika, starosti 18–79 godina, oba pola (33 žene i 19 muškaraca), obolela od dijabetesa melitusa tip 1 (35 ispitanika) i tip 2 (17 ispitanika), lečena u Institutu za endokrinologiju, dijabetes i bolesti metabolizma Kliničkog centra Srbije. Srednja starost ispitanika bila je 54,6 godina, a srednje trajanje dijabetesa 11,1 godina.

Kontrolna grupa formirana je od 67 dobrovoljaca iz Beograda, studenata Stomatološkog fakulteta Univerziteta u Beogradu, starosti 19–24 godine, oba pola (47 ženskih i 20 muških), bez parodontopatije i sistemskih bolesti.

Gingivni indeks (Löe-Silness) meren je sa vestibularne i mezijalne strane zuba, na šest donjih frontalnih zuba koji su najčešće bili prisutni kod bolesnika (sekstant broj 5 prema *World Health Organisation* – WHO). Praćena je promena boje, otok i krvarenje gingive; normalna gingiva okarakterisana je indeksom 0, blaga inflamacija – 1, umerena inflamacija – 2, jaka inflamacija – 3. Dobijene vrednosti podeljene su brojem zuba na kojima je izvršeno merenje (najčešće 6), da bi se dobila srednja vrednost za gingivni indeks sa vestibularne ili mezijalne strane [3].

Indeks dentalnog plaka (*Silness-Löe*) meren je na gingivnom delu vestibularne i mezijalne površine krunica istih šest zuba pomoću stomatološke sonde. Izražen je brojevima: 0 – nema plaka na površini krunice zuba; 1 – tanak sloj plaka na krunici zuba u blizini gingive, otkriva se sondom; 2 – umerena količina plaka na krunici zuba vidljiva golim okom; 3 – velika količina plaka koji ispunjava interdentalni prostor i gingivni, odnosno periodontni džep. Vrednosti plak indeksa sabrane su za svaku stranu zuba i podeljene brojem zuba na kojima je izvršeno merenje [3].

Dubina periodontnih džepova merena je pomoću graduisane stomatološke sonde (*University of Michigan*) i izražena u mm. Srednja vrednost dobijena je sabiranjem izmerenih dubina sa vestibularne, odnosno mezijalne strane i deljenjem brojem zuba na kojima je izvršeno merenje [3].

Korišćeni su sledeći statistički testovi: Kolmogorov-Smirnov test – za potvrđivanje normalne raspodele uzoraka bolesnika, Studentov *t* test – za upoređivanje pokazatelja stanja tkiva periodoncijuma kod dijabetesnih bolesnika u odnosu na zdravu populaciju i χ^2 test za ispitivanje stanja zuba dijabetesnih bolesnika zavisno od dužine trajanja dijabetesa, starosti, pola i nivoa oralne higijene. Korišćeni su programski paketi *Microsoft Excel*, SPSS za *Windows* i *Statgraf*.

Rezultati

Prema podacima za 2002. godinu u Beogradu je živelo 1 576 124 stanovnika. Registrovano je 3 806 stanovnika obolelih od dijabetesa melitusa, što čini 0,24% beogradske populacije. Procenat dijabetesnih bolesnika najveći je u opštinama u kojima živi stanovništvo najveće prosečne starosti [2].

U grupi obolelih od dijabetesa 15 bolesnika nosilo je totalnu protezu (oko 1/3 bezubih bolesnika), a 13 bolesnika imalo je manje od 10 zuba u vilici, što navodi na pretpostavku da mnogi dijabetesni bolesnici imaju uznapredovalu parodontopatiju.

U tabelama 1–3 prikazani su pokazatelji stanja tkiva periodoncijuma kod dijabetesnih bolesnika u odnosu na zdravu populaciju. Statistička značajnost razlika testirana je Studentovim *t* testom za nivo značajnosti $p < 0,05$. Očigledno je povećanje srednje vrednosti za plak indeks, gingivni indeks i dubinu periodontnih džepova sa vestibularne (V) i mezijalne (M) strane kod dijabetesnih bolesnika, što dokazuje da je parodontopatija česta komplikacija dijabetesa melitusa.

Samo u slučaju gingivnog indeksa vestibularno, dobijeno je da nema statistički značajne razlike između dve grupe.

Tabela 1

Indeks dentalnog plaka kod bolesnika sa dijabetes melitusom (DM) i parodontopatijom u odnosu na kontrolnu grupu

Ispitanici	Broj bolesnika	$\bar{x} \pm SD$	
		vestibularno	mezijalno
Kontrolna grupa	7	0,289 ± 0,2480	0,237 ± 0,2152
Bolesnici sa DM	11	1,164 ± 0,9684*	1,750 ± 0,6010*

*$p < 0,05$ u odnosu na kontrolnu grupu (Studentov *t* test)

Tabela 2

Gingivni indeks kod bolesnika sa dijabetes melitusom (DM) i parodontopatijom u odnosu na kontrolnu grupu

Ispitanici	Broj bolesnika	$\bar{x} \pm SD$	
		vestibularno	mezijalno
Kontrolna grupa	5	1,232 ± 1,0834	0,500 ± 0,6325
Bolesnici sa DM	11	1,055 ± 0,5318	1,394 ± 0,4087

*$p < 0,05$ prema kontrolnoj grupu (Studentov *t* test)

Tabela 3

Dubina periodontnih džepova (mm) kod bolesnika sa dijabetes melitusom (DM) i parodontopatijom u odnosu na kontrolnu grupu

Ispitanici	Broj bolesnika	$\bar{x} \pm SD$	
		vestibularno	mezijalno
Kontrolna grupa	8	0,0 ± 0,00	0,0 ± 0,00
Bolesnici sa DM	11	2,046 ± 1,0063*	2,709 ± 1,1879*

*$p < 0,05$ prema kontrolnoj grupu (Studentov *t* test)

Stanje zuba dijabetesnih bolesnika zavisno od dužine trajanja dijabetesa, starosti, pola, nivoa oralne higijene prikazano je u tabeli 4.

Tabela 4

Uticaj različitih faktora na broj i stanje zuba dijabetesnih bolesnika

Faktor rizika	Uticaj faktora rizika	χ^2	*p*
Dužina trajanja dijabetesa	negativan	21,072	< 0,05
Starost	negativan	12,140	< 0,05
Pol	nema	1,628	> 0,05
Oralna higijena	pozitivan	32,363	< 0,05

Ustanovljeno je da što dijabetes duže traje, bolesnici imaju manji broj prisutnih zuba u vilici, naročito ako boluju od dijabetesa duže od 20 godina.

Starost bolesnika ne utiče u tolikoj meri na broj prisutnih zuba, mada se, ipak, primećuje da broj zuba u vilici naglo opada posle 50. godine života.

Broj zuba je nezavisan od pola, a utvrđeno je da bolesnici sa lošom higijenom usne duplje imaju manji broj preostalih zuba od onih koji više vode brigu o higijeni.

Diskusija

Brojne epidemiološke studije dokazale su međusobnu povezanost dijabetesa melitusa i parodontopatije. Pokazano je da dijabetes predstavlja faktor rizika od nastanka i razvoja gingivitisa i parodontopatije, a stepen kontrole koncentracije glukoze u krvi značajan je činilac njihove povezanosti [6, 7]. Jedan od mogućih razloga za veću podložnost dijabetesnih bolesnika parodontopatiji su i povećana količina dentalnog plaka i smanjena količina salive u ustima (*xerostomia*) [8].

Bolesnici sa loše kontrolisanim dijabetesom starosti 45–64 godine imaju značajno povećanu prevalenciju teške parodontopatije u odnosu na zdrave istih godina, ali se ta razlika gubi sa starenjem i nije pronađena u starosnoj grupi 65–90 godina [9].

Pojedini autori pokazali su da na status periodoncijuma bolesnika sa dijabetesom tip 1 u velikoj meri utiču godine starosti [10]. Ako je, međutim, kompenzacija dijabetesa sa metaboličke i kliničke tačke gledišta dobra, ovi bolesnici imaju učestalost parodontopatije sličnu kontrolnoj grupi [11].

Na povezanost dijabetesa melitusa i parodontopatije utiču navike u ishrani, a u velikoj meri i briga o higijeni usne duplje [12]. Multivarijantne analize rizika pokazuju da dijabetesni bolesnici imaju oko tri puta veću šansu da obole od parodontopatije nego nedijabetičari iste starosti, pola i nivoa oralne higijene [6].

Za kliničku procenu stanja tkiva periodoncijuma kod dijabetesnih bolesnika često se koriste indeks plaka, gingivni indeks i dubina periodontnih džepova. Pokazano je da su ovi parametri značajno povećani kod obolelih od dijabetesa u odnosu na zdravu populaciju, što je u saglasnosti sa rezultatima dobijenim u ovom radu [13–16].

U gingivnoj tečnosti i krvi dijabetičara koncentracija glukoze je povišena u odnosu na nedijabetičare sa sličnim indeksom plaka i gingivnim indeksom, što uslovljava promene u okolnoj mikroflori i promene periodoncijuma kod dijabetesnih bolesnika [17].

Zbog svega navedenog parodontopatija se može uvrstiti u „klasične“ komplikacije dijabetesa. Sama parodontopatija, međutim, može imati značajan uticaj na metabolički status dijabetesnih bolesnika. Utvrđeno je da dijabetes tip 2 udružen sa parodontopatijom višestruko povećava rizik od smrti zbog ishemijske bolesti srca, dijabetesne nefropatije i kardiorenalnih bolesti uopšte [18].

Lečenje parodontopatije kod dijabetesnih bolesnika ima povoljne efekte na kontrolu glikemije i samim tim na poboljšanje opšteg stanja ovih bolesnika [19–21].

Studije su pokazale da tretiranje zubnog kamenca sa ili bez prateće antibiotske terapije (doksiciklin, amoksicilin i klavulanska kiselina) značajno poboljšava stanje periodontnog tkiva, sa manjim ili većim uticajem na nivo kontrole glikemije (praćene merenjem nivoa HbA1c) [22–28].

Zaključak

Pokazatelji nivoa oralne higijene i stanje periodontnog tkiva (indeks dentalnog plaka, gingivni indeks, dubina periodontnih džepova) značajno su pogoršani kod dijabetesnih bolesnika. Negativan uticaj na broj preostalih zuba imaju starost (> 50 godina), dužina trajanja dijabetesa (> 20 godina) i loša oralna higijena. Polovinu ispitanih bolesnika zbog gubitka svih ili velikog broja zuba nosi parcijalnu ili totalnu protezu, što pokazuje da je parodontopatija značajna komplikacija dijabetesa melitusa.

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Etiology of ischemic stroke among young adults of Serbia

Etiologija ishemijskog moždanog udara kod mlađih odraslih osoba u Srbiji

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Abstract

Background/Aim. Etiology of ischemic stroke (IS) among young adults varies among countries. The aim of the study was to investigate the causes and risk factors of IS in the young adults of Serbia. **Methods.** A total of 865 patients with IS, aged 15 to 45 years, were treated throughout the period 1989–2005. Etiologic diagnostic tests were performed on the patient by the patient basis and according to their availability at the time of investigation. The most likely cause of stroke was categorized according to the TOAST (Trial of ORG 10172 in Acute Stroke Treatment) criteria. **Results.** There were 486 men and 379 women, with 19% of the patients ≤ 30 years old. Large artery arteriosclerosis and small artery disease were confirmed in 14% of the patients, and embolism and other determined causes in 20%. Undetermined causes made up 32% of the patients, mostly those (26%) with incomplete investigations. Smoking (37%), hypertension (35%) and hyperlipidemia (35%) were the most common risk factors. Rheumatic heart diseases and prosthetic valves were the most common causes of IS. Arterial dissections and coagulation inhibitors deficiency were detected in a small number of patients. **Conclusion.** Etiology of IS among Serbian young adults shares characteristics of those in both western and less developed countries.

Key words:
brain ischemia; adolescent; adult; risk factors; yugoslavia.

Apstrakt

Uvod/Cilj. Etiologija ishemijskog moždanog udara (IS) kod mladih razlikuje se u različitim zemljama. Cilj ovog rada bio je istraživanje faktora rizika od IS kod mlađih odraslih osoba u Srbiji. **Metode.** Ukupno 865 bolesnika sa IS, starosti 15–45 godina, lečeno je u periodu 1989–2005. Etiološki dijagnostički testovi izvršeni su na bazi bolesnik–po–bolesnik i prema tome koliko ih je bilo u vreme istraživanja. Najčešći uzrok udara svrstan je prema kriterijumu *Trial of ORG 10172 in Acute Stroke Treatment* (TOAST). **Rezultati.** Bilo je ukupno 486 muškaraca i 379 žena, a od toga 19% bolesnika starosti ≤ 30 godina. Ateroskleroza velikih arterija i oboljenje malih arterija potvrđeni su kod 14% bolesnika, a embolija i drugi uzroci kod 20% od ukupnog broja bolesnika. Kod 32% bolesnika nisu određeni uzroci, kod većine njih (26%) zbog nepotpunosti podataka. Najčešći faktori rizika bili su pušenje (37%), povišeni krvni pritisak (35%) i hiperlipidemija (35%). Najčešći uzroci IS bili su reumatska oboljenja srca i veštački zalisci. Kod malog broja bolesnika nađeni su arterijska disekcija i nedostatak inhibitora koagulacije. **Zaključak.** Etiologija IS kod mlađih odraslih osoba u Srbiji ima iste karakteristike kao u zapadnim i manje razvijenim zemljama.

Ključne reči:
mozak, ishemija; adolescenti; odrasle osobe; faktori rizika; srbija.

Introduction

Ischemic stroke (IS) among young adults is a rare event that makes 5–10% of all stroke patients [1–3]. Also, the causes of IS in the young differ among various countries and from those in older population. Cardioembolism and other determined causes, mostly arterial dissections, were reported as the main causes of IS among young adults [1, 4–12]. Large artery atherosclerosis and small artery disease had rarely outnumbered other defined causes of stroke [2, 13–16]. However, the atherosclerosis is the main etiologic factor among the young of developing countries, as it used to be in developed countries more than two decades ago.

The aim of this study was to find out if risk factors and causes of IS among the young adults of Serbia are similar to those in other countries.

Methods

This prospective study included a total of 865 consecutive patients, 15–45 year old, with first ever transitory ischemic attack (TIA) or completed IS treated in the Department for Emergency Neurology from January 1989 to December 2005. In 1989, initially as a part of the Master thesis, a research project investigating etiology of stroke in young adults was started and continued until today. Clinical diagno-

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sis of IS was confirmed by brain computed X-ray tomography (CT) in 834 of the patients, brain magnetic resonance imaging (MRI) in 21 of the patients, brain nuclear scintigraphy in 7 and in 3 of the patients by autopsy.

Hypertension, diabetes mellitus, hyperlipidemia, cigarette smoking, previous TIA or stroke, heavy alcohol consumption, drug abuse, previous history of migraine, current oral contraceptive use and family history of stroke among first- and second-degree relatives were considered as risk factors for IS. Hypertension was regarded present when a patient had previously been advised to take antihypertensive drugs, or when blood pressure was > 140/90 mmHg in two different occasions at least 7 days after the stroke onset. Diagnosis of diabetes mellitus was established if a patient had already been treated or according to the criteria of the World Health Organization (WHO) [17]. Hyperlipidemia was considered risk factor when fasting blood total cholesterol was > 6.2, LDL - cholesterol (LDL-C) was > 4.2 mmol/l or HDL - cholesterol (HDL-C) < 1.1 or triglycerides were > 1.7 mmol/l [18]. Current cigarette smoking was verified when a patient had smoked > 5 cigarettes per day at least for one year, and as an ex-smoker a patient who had stopped smoking more than one year ago. Alcohol consumption was regarded chronic heavy drinking when a patient regularly took > 3 heavy drinks per day (> 36 g of alcohol/day) and as an acute alcohol intoxication when a patient had taken > 48 g of alcohol during preceding 24 hours [19]. Migraine was defined according to the criteria of International Classification of Headache Disorders [20]. Oral contraceptives were considered risk factor if they were used at any time in a 3-month period before the stroke [21]. Heart diseases were categorized as high-risk or low-risk cardio embolic diseases [22, 23]. Low-risk cardioembolic diseases were considered potential cause of stroke only in the absence of other more possible etiological factors.

The diagnostic protocol for young IS patients included medical history, cardiac and neurological examinations, assessment of risk factors and appropriate laboratory tests. Routine laboratory tests, ophthalmoscopic exam, electrocardiogram (ECG) and chest X-ray radiography were done in nearly all the patients. Other diagnostic tests for identifying etiology of IS were performed according to the patient by the patient selection. For example, detailed coagulation or immunological laboratory tests or angiography were not performed in the presence of high risk cardioembolic diseases or an echocardiography was not mandatory for the patients with normal cardiac physical findings and high grade carotid stenosis concurrent with infarct localization. During this 17-year long period not all of the contemporary diagnostic tests were accessible and some of the routine diagnostic procedures were unavailable during the period of economic sanctions. Transthoracic echocardiography (TTE) was done in 451 of the patients (52%) and transesophageal (TEE) in only 38 of the patients. Vascular examination was done in 458 patients (53%) of which 289 ones were receiving cerebral or MR angiography (MRA) and 268 ones Doppler ultrasound exams. Blood lipids were analyzed in 412 of the patients (48%). Immunological testing (antinuclear antibodies, anti-DNA, antineutrophil cytoplasm antibodies, immune complexes, C3, C4, VDRL) was done in 375 of the patients (43%), antithrombin III (AT III) in 81 of the patients, protein C (PC) in 67, protein S (PS) in 32, lupus anticoagulant (LAC) in 107, anticardiolipin antibodies (ACA) in 98 and homcistein in 26 of the patients. Routine and immunological cerebrospinal fluid testing were done in 417 (48%) and 285 (32%) of the patients, respectively.

Initially, most likely cause of stroke was categorized as atherosclerotic disease, cardioembolism, nonatherosclerotic arteropathy, haematological disorder or undetermined cause according to previously described definitions [22]. Later, we revaluated the data and used the TOAST criteria to reassign the most likely cause of IS into one of 5 groups: 1) large artery atherosclerosis (LAA), 2) small artery disease (SAD), 3) embolism (EMB), 4) other determined causes (ODC), and 5) undetermined cause (UDC) with distinguishing unknown, uninvestigated and a multiple causes subgroup [23].

For the statistical analysis, data were stored on SPSS version 13.0 software. Statistical evaluation was made by means of t-test and one-way ANOVA for numerical variables and χ^2 and ANOVA tests for proportions with confidence intervals. Multinominal logistic regression was performed for the evaluation of a possible effect of age, sex and risk factors on stroke subtypes. $P < 0.05$ was considered statistically significant.

Results

There were 379 women and 486 men with IS. Table 1 shows main demographic characteristics and risk factors in different subtypes of IS. Mean age of women (36.7±7.3) was significantly lower than that of men (37.6±7.0) ($p = 0.045$, 0.98, 95%CI: 0.02-1.94). In a group of patients with age up to 30 years women predominated (53%) and after that age men were more affected (58%). Carotid area was involved in 80% of patients, and 9% of patients had TIA.

Among hypertensive patients only 50% of them had been treated before the stroke and among those who did not take antihypertensives nearly 66% of the patients knew that they had hypertension for more than 5 years. Twenty-five out of 74 patients had insulin-dependent type of diabetes mellitus. Fifty-six patients had increased triglycerides, 18 high total cholesterol, 55 patients had both disorders and 16 patients had alone HDL-C decrease. Of the current smokers 93% smoked more than 10 cigarettes per day.

Univariate statistical analysis showed significantly higher presence of all traditional risk factors in LAA group (Table 1). Multinominal logistic regression showed significantly more often presence of hypertension (OR 4.6, 95%CI: 2.3-9.2) and hyperlipidemia (OR 2.5, 95%CI:1.3–5.0) in LAA group. Hypertension (OR 5.1, 95%CI:2.4–10.9) and hyperlipidemia (OR 2.3, 95%CI:1.1–4.9) were also more frequently present in the SAD group. Also, the age of < 30 years was more common in the ODC group (OR 1.9, 95%CI:1.0–3.6).

The angiography, carried out on the average 10 days after the stroke onset, showed pathological findings in 77% of all the investigated patients. In 75 patients of the LAA group who received angiography significant intracranial ste-

Table 1

Demographic characteristics and risk factors in stroke subtypes among 865 young adults

Demographic caracteristics	TOTAL (n)	LAA	SAD	EMB	ODC	UDC	*p**
Number (%)	865 (100)	117 (14)	120 (14)	174 (20)	173 (20)	281 (32)	
Age (y, mean±SD)	37.2±7.1	39.4±5.3	39.5±5.5	36.9±7.3	32.8±7.8*	38.2±6.6	< 0.001
Age ≤ 30 y (%)	19	7	9	21	38*	15	< 0.001
Male sex (%)	56	69*	60	49	49	58	= 0.002
Hypertension (%)	35	60*	74*	19	10	35	< 0.001
Diabetes mellitus (%)	9	19*	12	5	1	10	< 0.001
Hyperlipidemia (%)	35	59*	56	17	25	31	< 0.001
Previous transient ischemic attack/stroke (%)	23	30	27	20	23	22	= 0.250
Smoking (%)	37	51*	42	24	33	38	< 0.001
Alcohol abuse (%)	6	17*	5	2	3	5	< 0.001
Family stroke (%)	9	10	12	6	6	11	= 0.172

LAA – large artery atherosclerosis, SAD – small artery disease, EMB – embolism, ODC – other determined causes, UDC – undetermined cause

nosis was present in 65% of them. Duplex ultrasound examination of cervical arteries discovered hemodynamically significant stenotic changes in only 15% of the examined patients.

High-risk embolic heart diseases were found in 59% of the patients with cerebral embolism (Table 2). Overall, there were 37 patients with atrial fibrillation and 12 patients with intracardial thrombus. Four out of 26 patients with rheumatic heart disease (RHD) were not aware of its presence until stroke onset. Among 14 patients with RHD and atrial fibrillation, eight had not received antithrombotic drugs before the stroke onset, 2 received antiplatelet agents and only 4 pa-

Table 2

Sources of cerebral embolism in 174 patients

Sources (risk conditions)	Number of patients
*High risk embolic heart conditions**	103 (59%)
Dilated cardiomyopathy	29
Rheumatic heart disease†	22
Prosthetic valves	20
Infective endocarditis‡	8
Heart tumour	7
Acute myocardial infarction	5
Akinetic left ventricular segment or aneurysm with ICT	4
Marantic endocarditis with MVP and ICT	1
Hypertrophic cardiomyopathy with AF and/or ICT	3
MVP with AF and/or ICT	3
Atrial septal aneurysm and MVP with ICT	1
Low risk embolic heart conditions	63 (36%)
Mitral valve prolapse§	18
Atrial septal aneurysm‖	15
Hypokinetic left ventricular segment	9
Paradoxical embolism	6
Lone atrial fibrillation	4
Marantic endocarditis	3
Aortic valve prolapse	2
Mitral annulus calcification	2
Other	4
Other sources	8 (5%)
Ascending aorta sclerosis	3
Distal embolism from MCA aneurysm	2
Distal embolism after cervical ICA surgery	2
Air embolism	1

ICT – intracardial thrombus; MVP – mitral valve prolapse; AF – atrial fibrillation; PFO – patent foramen ovale *in 37 patients also AF; †14 with AF or ICT; ‡ 4 with rheumatic heart disease; 2 with MVP and one heroin addict; § one with PFO; ‖3 also PFO and 2 MVP

tients had taken anticoagulants but with initial prothrombin time beyond therapeutic level in 3 of them. Also, the initial prothrombin time was in the therapeutic range in only 7 of 20 patients with mechanical prosthetic valves. One patient with mechanical valve had been treated with aspirin and another had not received any antithrombotic therapy. Mitral valve prolapse (MVP) was present in overall 27 of the patients and atrial septal aneurysm in 16. TTE showed pathological findings in 51% of all the examined patients. In 38 patients with previously normal TTE, subsequently performed TEE discovered abnormal findings in 27 of them, mostly atrial septal aneurysm or MVP.

In the ODC group the nonarteriosclerotic arteropathies were diagnosed in 56% of the patients (Table 3). In 8 patients, cerebral angiographic findings with clinical signs, blood and CSF results suggested the diagnosis of possible isolated CNS angiitis. Among the viral cerebral vasculitides CSF immunological tests confirmed HIV or Epstein-Barr virus infection in two patients each and varicella zoster and herpes simplex type 1 in one patient. In one patient common viral antibodies were not detected.

Primary antiphospholipid syndrome (APL) was confirmed in 7 patients as a cause of IS and secondary one in three. Among 107 tested patients, LAC was present in six with two of them having systemic lupus erythematosus (SLE). Low- or medium-high positive ACA were detected in 13 of 98 tested patients. Six of these patients had primary APL syndrome, two had SLE and one Sjögren disease. In

Table 3

Other determined causes of ischemic stroke in 173 young adults

Causes of ischemic stroke		Number of patients
Nonatherosclerotic noninflammatory arteropathies		63 (36%)
Moyamoya		15
Arterial dissection	(spontaneous 7, traumatic 7*)	14
Vasospasm	(eclampsia 4, PBA 2, migraine 2, marijuana 2, HTE 1, SAH 1, unknown 1)	13
Fibromuscular dysplasia		9
Congenital intracranial artery stenosis		4
Double artery kinking		3
M. Burger		2
Lateral wall compression		2
Arterial intimal hyperplasia†		1
Nonatherosclerotic inflammatory arteropathies		35 (20%)
Isolated central nervous system angiitis		8
Viral-induced		7
Undefined systemic vasculitis		5
Systemic lupus erythematosus vasculitis		5
M. Behçet		2
Drug induced		3
Bacterial meningitis		2
Sneddon syndrome		2
Anti-neutropil cytoplasmic antibodiesANCA vasculitis		1
Haematological causes		37 (22%)
Antiphospholipid syndrome		10
Disseminated intravascular coagulationDIC and/or sepsis		7
Coagulation inhibitor deficit		6
Significant hemorheological changes		5
Thrombocytosis		2
Thalassemia		1
Non-defined thrombophillia		6
Other causes or mechanism not defined		38 (22%)
Pregnancy/puerperium		14
Systemic lupus erythematosus		9
Presumed hemodynamic mechanism		9
Acute alcohol intoxication		2
Hyperhomocisteinemia		2
Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke		1
Iatrogenic – placement of central venous cannula		1

PBA – postpartum benign arteropathy; HTA – hypertensive encephalopathy; SAH – subarchnoidal haemorrhage *one with positive ACA; †autopsy confirmed, oral contraceptives during 8 years

two of the patients with positive ACA the cause of IS was posttraumatic arterial dissection in one and the presence of intracardial thrombus in another one. One patient with positive ACA who had MVP with thickened valves was classified to embolic type of stroke and in one woman postpartum benign angiopathy was responsible for the stroke onset. Decreased AT III was present in 5 of 81 and decreased PC in 4 of 67 tested patients. Protein S was normal in all 32 tested patients. Hereditary thrombophillia was confirmed in one patient with AT III deficiency and in another one with both AT III and PC deficit. In one puerperal woman with low values of activated PC resistance who developed fatal MCA stroke and acute myocardial infarction genetic investigation was not done.

Overall, there were 43 women who had stroke during pregnancy or immediately postpartum. In 14 of them no other possible cause of stroke was detected. Only two patients out of 66 with migraine had possible migraine-induced stroke. Current oral contraceptive use was recorded in only 10 women, and in one woman who had taken them for 8 years the autopsy revealed signs of arterial intimal hyperplasia and complete carotid occlusion. In 3 of the patients the onset of stroke was related to marijuana or heroin abuse.

Discussion

This is one of the largest published series of IS in young adults reported from a single medical center. This fact remains even if we exclude patients with TIA and all patients without complete investigation. Also, this study was one of the longest prospective studies that investigated causes of IS among young adults using predesigned protocol. However, for the most of the study period (1991–2000) our country was exposed to the civil war and economic sanctions that made up diagnostic facilities becoming poorer than in the beginning of the study period. For these reasons, a small proportion of our patients received vascular examination, TEE and update coagulation studies compared with other similar studies [2, 6, 8, 10, 12, 14, 24]. Although our study was prospective, lack of all necessary diagnostic procedures during this period resulted in 26% of the incompletely investigated patients. In the Canadian study such patients made up 22% and in a French one only 9.5% of all the patients [10, 24].

The demographic characteristics and presence of hyperlipidemia and smoking were similar to those of other western countries and the frequencies of hypertension and diabetes mellitus were closer to those in Asian studies (Table 4) [1, 2, 9, 13, 14, 24, 25]. In addition, LAA and SAD groups made up one-quarter of all patients, which is higher than in most western studies and less than in Middle or Far East countries (Table 4) [1, 2, 5, 6, 10, 12, 14, 15, 24, 26]. We believe that our proportion of LAA was underestimated because this diagnosis requires a positive finding of significant artery stenosis. Among our patients without full investigation there were even 73 of them who had at least two major risk factors for stroke, as

Table 4

Demographic characteristics, risk factors and causes of ischemic stroke (IS) in the young less than 45 years old in different studies

Country	Pat. No.	HT (%)	SM (%)	DM (%)	HL (%)	Stroke subtypes (%)				
						LAA	SAD	EMB	ODC	UND
Italy[24]	394	23	56	2	Total 15	5.4	2.5	33.7	28	23.8
Switzerland[5]	202	7.9	46.0	1.5	Ch 8.9	5.4	2.5	21.4	46.0	22.8
Sweden[1]	107	23.1	36.2	2.7	Ch 7.7, T 9.5	12.1	4.7	32.7	29.9	20.6
France[21]	296	18.6	55.1	14.2	Total 37.5	8.4	7.1	8.7	25.7	50.0
USA,Iowa[6]	329					9.7	7.9	17.6	30.4	34.3
USA,Indiana[9]	116					16.4	2.6	13.8	43.9	23.2
Canada[10]	356					5.9	7.6	14.3	27.5	44.6
Saudi Arabia[15]	70					12.9	24.3	17.1	30.0	15.7
Korea[2]	149	38.3	51.0	10.1	Total 8.1	20.8	17.4	18.1	26.8	16.8
Taiwan[14]	241*	45.8	49.8	14.8	Total 53.1	7.9	22.4	19.5	24.5	25.7
Present study	865	35.4	36.6	8.6	Total 30.3 Ch 17.7, T 26.9	13.5	13.9	20.1	20.0	32.5

HT – hypertension; SM – smoking, DM diabetes mellitus; HL – hyperlipidemia; Ch – cholesterol; T – triglycerides; *transitory ischemic attack excluded; LAA – large artery atherosclerosis; SAD – small artery disease; EMB – embolism; ODL – other determined causes; UND – undetermined cause.

In the UDC group the cause of stroke was not discovered in 52 of the patients (6%) despite complete investigations. In only six patients more than one possible mechanism of stroke was detected. There were 223 patients (26%) without complete investigations. In 102 of these patients neuroimaging showed large-artery brain infarction and lacunes in 31. There were 72 patients who had 2 or more major risk factors for stroke.

well as CT findings of large artery infarction. It is highly possible that a substantial number of these patients would have had LAA if more vascular examinations had been done.

Totally 14% of SAD patients is higher than the most of western studies reported [1, 5, 6, 9, 10, 12, 24]. This may well be the consequence of the inadequate preventive measures and poor control of risk factors during the past years. The fact that even half of our patients with hypertension had not received antihypertensive treatment before their IS and that of these as many as two thirds knew that they had hypertension for at least 5 years was the best illustration of an improper health education and poor medical care.

The proportion of cerebral embolism in our study was not different from the most recent studies except that RHD or prosthetic valves made up as much as a quarter of all these patients (Table 3) [2, 6, 9, 10, 14, 15]. During previous years, poor facilities for detection and follow-up of patients at risk resulted in an inadequate control of RHD and anticoagulation level. Only 4 out of 14 patients with RHD and AF received anticoagulants before the stroke onset. Lacking of preventive programs was also reported in India and Mexico where RHD and prosthetic valves made up as high as two thirds of all cardioembolic strokes in young adults [7, 27]. In most other studies MVP, patient foramen ovale (PFO) and, atrial septal aneurysm (ASA) were common sources of cardiac embolism [1, 6, 9, 10, 12–14]. In our series these heart conditions were undiagnosed because of the low rate of the applied TTE and TEE. Out of only 38 patients who underwent TEE, in 27 were detected related pathological findings. This confirmed the importance of TEE in discovering potential causes of IS in the young as was documented in many other studies [1, 2, 10, 12, 28, 29]. However, some of these abnormalities are common in the general population and detecting them does not mean that they had a causal role in the stroke. It is advisable to perform a full vascular examination and to exclude more probable causes of stroke in these patients.

The ODC was reported as the most common subtype of IS in the young (Table 4) [2, 5, 6, 9, 10, 14, 15, 24]. In our series this group of patients it was as common as embolism, which was also found by some other authors [1, 25] . In nearly all recent studies an arterial dissection was the most often detected separate cause of IS in young people, with a frequency of 14 to 20% of performed cerebral angiograms [2, 10, 12, 14, 24]. The proportion of arterial dissections among angiograms (3.5%) in our study was more in accordance with some earlier studies when they were not so readily recognized and made up 0.4 – 10.1% of angiographic findings [4, 6, 8, 13]. Nowadays, cross-sectional MRI in combination with MRA is the method of choice for initial diagnosis of craniocervical artery dissections [30]. A low proportion of arterial dissections in our study may imply inexperience of our clinicians and neuroradiologists in detecting this entity, but even more it reflects the unavailability of MR neuroimaging during the past years. Moyamoya disease was detected in 5% of the performed angiograms and this was closer to the findings of Far East studies than to those of western countries [2, 10, 13, 14, 24]. It is not quite clear to us what were the reasons for this higher proportion of Moyamoya disease among our patients. However, Adams et al. [6] also reported higher appearance of this cause in the young with IS.

We confirmed a haematological disorder as a cause of IS in 4.3% of all our patients. Others reported this proportion being 4.1 – 5.7% [6, 10, 24]. ACA were positive in 13% of those tested and in 7 patients we regarded APL syndrome as a cause of IS. In the selected groups of young patients with IS, positive ACA were detected in up to 23% of patients [2, 7, 14, 31, 32]. However, some studies with a low frequency of ACA even questioned the relevance of their role in unselected young stroke patients [1, 6, 33]. This notion prevails even more for a hereditary deficiency of coagulation inhibitors [32]. A systemic review of the literature showed that the prevalence of inherited deficiencies of PC, PS and AT III was low in unselected IS patients. Even in young stroke patients deficiency of coagulation inhibitors was rare and mostly of the acquired type [1, 2, 6, 34]. Only in a few studies of young stroke patients inherited deficiencies of coagulation inhibitors were reported with frequencies of 4 – 7.5% [12, 14, 35]. Out of a small number of the tested patients, we documented hereditary deficit of AT III in two patients, of whom one also had PC deficit, and very low APCR ratio in one puerperal woman whose death precluded genetic analysis for Factor V Leiden mutation.

Conclusion

Our study on young ischemic stroke patients is one of the largest reported, showing that our patients did not differ in their demographic characteristics from western stroke patients. Similar to the patients of Middle or Far East, our young people had higher proportion of hypertension and lacunar strokes. Embolism and other defined causes were the most common etiologic subtypes of IS, but still with very high proportion of rheumatic valvular diseases. Arterial dissections and coagulation disorders might have been underestimated. Migraine, oral contraceptive use and drug abuse were rarely associated with IS in our study. It is our assumption that the poor diagnostic facilities, low health education and inadequate preventive measures during past years are responsible for some of the differences. During the last few years, there have been some indications that the etiology of stroke in the young of Serbia is more similar to the reports of developed countries.

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Immunohistohemical evidences of pregnancy in uterine curettage tissue by the use of a double immunocytochemical staining technique using cytokeratin 7 and vimentin antibodies

Imunohistohemijsko dokazivanje trudnoće u kiretiranom tkivu uterusa pomoću tehnike dvostrukog imunocitohemijskog bojenja primenom antitetila na citokeratin 7 i vimentin

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Abstract

Background/Aim. Usual histopatological diagnosis of intrauterine pregnancy is made by demonstration of chorionic villi, but in the curettage tissue from intrauterine miscarriage they may not be present in all cases. The use of monoclonal antibody against cytokeratin as a sensitive and reliable marker for the morphologic discrimination between invasive trophoblastic (IT) cells and decidual cells has been well established. The aim of this study was to determine the presence of pregnancy in endometrial curettings when chorionic villi are absent from patients suspected of intrauterine pregnancy. **Methods.** Twenty cases of endometrial tissue specimens were investigated for cytokeratin and vimentin expression by a double immunostaining for detection of IT cells. **Results.** Out of the total number of cases (20) 17 cases expressed cytokeratin 7 positive IT cells, that are an evidence of pregnancy. **Conclusion.** The obtained results indicated, that double immunohistochemical demonstration of cytokeratin and vimentin is useful for identifying pregnancy in all chorionic villi-negative cases.

Key words:
abortion, spontaneous; immunohistochemistry; vimentin; keratins; trophoblasts.

Apstrakt

Uvod/Cilj. Patohistološka dijagnoza intrauterine trudnoće potvrđuje se prisustvom horionsnih čupica, ali one ne moraju biti prisutne u svim uzorcima kiretiranih tkivnih uzoraka endometrijuma. Korišćenjem monoklonskih antitela za citokeratin kao pouzdanih i osetljivih markera za morfološko razlikovanje invazivnih trofoblastnih ćelija i ćelija decidue u širokoj je upotrebi. Cilj ovog rada bio je da se utvrdi prisustvo trudnoće u slučajevima suspektne materične trudnoće, kada u kiretažnom materijalu nisu nađene placentne čupice. **Metode.** Kod 20 tkivnih uzoraka endometrijuma ispitivana je ekspresija citokeratina 7 i vimentina primenom dvostrukog imunološkog bojenja. **Rezultati.** Od ukupno 20 uzoraka 17 su pokazali ekspresiju citokeratin 7 pozitivnih ćelija invazivnog trofoblasta, što je znak evidentne trudnoće. **Zaključak.** Dvostruko imunohistohemijsko dokazivanje citokeratina 7 i vimentina može biti od značajne koristi u utvrđivanju prisustva trudnoće kada su placentne čupice otsutne.

Ključne reči:
abortus, spontani; imunohistohemija; vimentin; keratin; trofoblasti.

Introduction

The usual histopathologic diagnosis from intrauterine pregnancy is made by demonstration of the chorionic villi. Chorionic villi may not be seen in the curettage material of intrauterine miscarriage tissue in all cases. The presence of trophoblast in uterine curetting specimen is also an evidence of pregnancy. Although the morphology of trophoblast has been studied extensively and well described, recognition of invasive trophoblast (IT) cells intermingled with the decidual cells may be difficult, because a subset of the polyhedral IT cells is morphologically very similar to the decidual cells [1].

The use of monoclonal antibody against cytokeratin as a sensitive and reliable marker for the morphologic discrimination between IT cells and decidual cells has been well established [2].

For trophoblast, usually employed markers are the presence of cytokeratin 7, and the absence of vimentin. In contrast, for decidual cells are characteristic positive expression of vimentin and the absence of cytokeratin 7 [3].

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In this study we presented a double immunoenzymatic labelling to distinguish IT cells and decidual cells simultaneously in the same tissue sections.

Methods

The endometrial curettage material was obtained from 20 patient clinically suspected of having miscarriage, but with no chorionic villi in curettage tissue. We had two control groups. The positive control included of 10 patients with chorionic villi in their endometrial curettage material, and the negative control of 10 patients with uterine curettage for menstrual irregularities. The material was fixed in 10% buffered formalin, routinely processed, embedded in paraffin, cut and stained with haematoxylin-eosin (HE) and PAS.

Double immunostaining was performed as follows: the deparaffinized tissue sections were boiled in citrate buffer, pH 6.0, for 5 minutes, 3 times in microwave oven. The sections were first incubated with cytokeratin antibody (one part sections) and with vimentin antibody (second part sectons) in humidifed chamber at 4 °C overnight followed by PAP immunoperoxidase. The immunoreactivity was detected using 3-amino-9-ethylcarbazole (AEC) chromogen (red) [4].

After five washes in tris-buffered saline, the slides were incubated with vimentin antibody (one part sections) and with cytokeratin antibody (second-part sectons) at 37 °C for 60 minutes follwed by APAAP method [5]. The immunoreactive sites were detected with fast blue BB chromogen (blue). Finally, slides were mounted with an aqueous medium.

As control we used the alkaline phosphatase antialkaline phosphatase (APAAP) method. Primary monoclonal antibodies (cytokeratin 7 and vimentin) were incubated, after epitope retrieval with citrate buffer, in humidifed chamber at 4 °C overnight. Secondary and tertiary immunoreactions were performed at room temperature for 60 minutes. The antibody-antigen complexes were visualized by incubation for 20 minutes in new-fuchsin substrate (red). The sections were counterstained with haematoxylin and mounted in glycerol gelatine.

The antibodies and all other reagents were from DAKO, Glostrup, Denmark.

Results

Besides the characteristic growth pattern, IT cells are often difficult to recognize, because they closely resemble decidual cells on slides stained with HE or PAS (Figure 1).

The discrimination of decidual cells and IT cells is not difficult by the use of immunohistochemical staining of cytokeratin 7 and vimentin. The cytokeratin 7 immunoreactivity characterized IT cells as red intracytoplasmatic staining with new-fuchsin as chromogen (Figure 2). The IT cells as endovascular trophoblast were embeded in the wall of spiral artery as intramural trophoblast. The endovascular trophoblast intensively stained with anti-cytokeratin antibody were in contrast to the decidual cells which were cytokeratin negative (Figure 3). The immunostaining with antivimentin antibody as a marker for mesenchymal cells showed strong staining of decidual stromal cells, whereas glandular cells showed no vimentin expression (Figure 4).

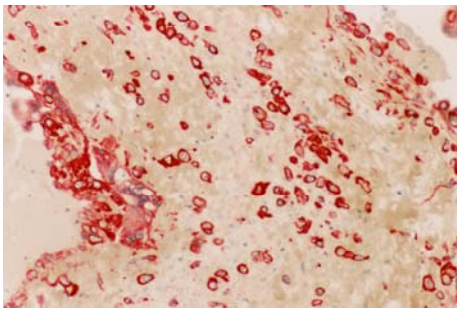

Fig. 1 – Invasive trophoblast cells are indistinct from decidual cells, a spiral artery in the center of the field (PAS; × 200)

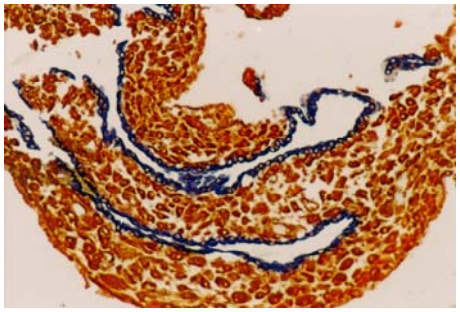

Fig. 2 – Strong cytokeratin 7 positive invasive trophoblast cells (APAAP; × 200)

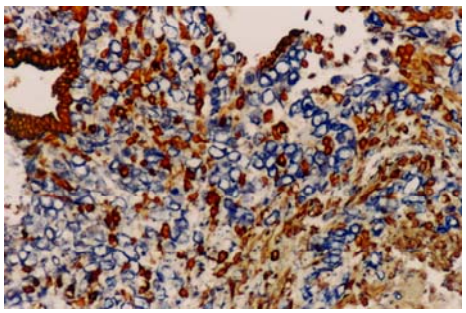

Fig. 3 – Endovascular intramural trophoblast, cytokeratin (APAAP; × 200)

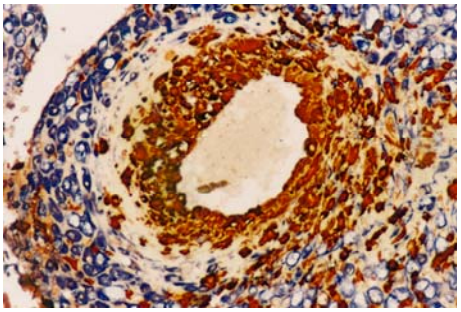

Fig. 4 – Strong vimentin positive decidual cells, glandular cells – negative (APAAP; × 200)

By the immunoenzymatic labelling two antigens (cytokeratin 7 and vimentin) localized in different cellular compartment (glandular and decidual cells) with two different

chromogen methods, we observed two separate colours (Figure 5). The IT cells occupying the cross sections of the vessel wall, demonstrated strong cytokeratin staining. The vimentin positive staining were present in decidual cells around spiral aretries (Figure 6).

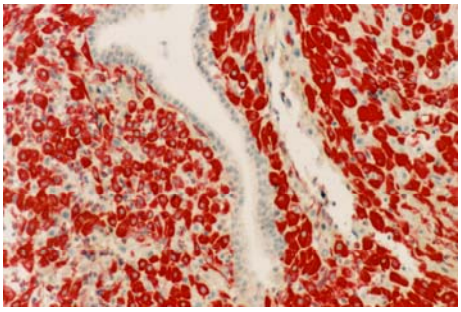

Fig. 5 – Double immunostaining: cytokeratin – endometrial glands (blue), vimentin – decidua (red) (PAP/APAAP; × 200)

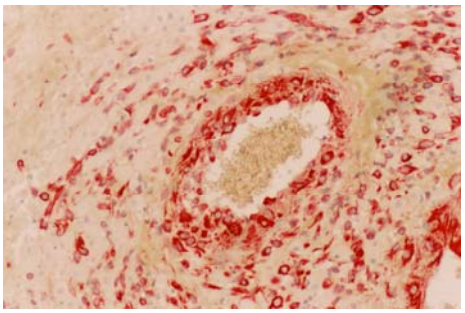

Fig. 6 – Double immunostaining: a spiral artery in the center of the field, invasive trophoblast cells cytokeratin-positive (red), decidual cells vimentin-positive (blue) (PAP/APAAP; × 200)

The cytokeratin immunostains both endometrial gland lining and scattered type of interstitial IT cells. The cytokeratin positive interstitial type of IT cells were dispersed among vimentin-positive decidual cells and formed a defined mosaic pattern (Figure 7).

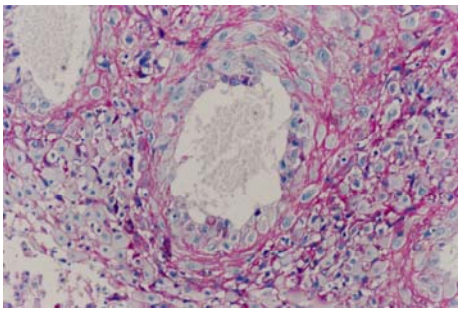

Fig. 7 – Double immunostaining: cytokeratin (red) positive interstitial type invasive trophoblast cells are dispersed among vimentin (blue) positive decidual cells (PAP/APAAP; × 200)

With double immunostain we identified IT cells in 17 out of 20 samples of endometrial curettage without chorionic villi.

Discussion

In the absence of chorionic villi, unequivocal trophoblastic cells are a convincing proof of pregnancy. Distinguishing trophoblast cells from decidual cells on morphologic grounds could be difficult.

Traditionally, two types of trophoblasts have been described: cytotrophoblast and syncytiotrophoblast.

Subsequent light microscopic, histochemical, electron-microscopic studies and immunocytochemical investigations have confirmed the presence of an invasive form of trophoblastic cells with characteristic morphologic and biochemical features. This third type of cells has been designated as invasive or intermediate trophoblast (IT) [6].

The first clear marker of an invasive trophoblast was described by Kurman et al [7], who demonstrated that first-trimester invasive trophoblasts react with anti-human placental lactogen antibodies. They coined the term "intermediate" invasive trophoblasts, partly because of their intermediate size between cyto- and syncytiotrophoblast.

Within implantation site of decidua several subsets of IT cells are present: interstitial trophoblast dispersed within decidua, and endovascular trophoblast which invades spiral aretries in the endometrium and myometrium, modifying them into noncontractile tubes allowing a stedy flow of maternal blood into the sinusoids [8]. The intravascular implantation site IT cells, formed cohesive cell aggregates in the wall and lumen of spiral aretries, demonstrated strong cytokeratin staining [9].

The utility of monoclonal antibody against cytokeratin for the morphologic discrimination between IT and decidual cells that is higher sensitivity compared to other immunostains has been well established. Anticytokeratin 7 antibody reacts with the 54 kDa cytokeratin intermediate filament protein, and it is shown in most glandular and ductal epithelia [10].

The decidua is a heterogeneous tissue which comprises not only the typical swollen stromal cells but also glands, blood vessels and numerous infiltrating cells. The decidual stromal cells are of mesenchymal origin. Antivimentin antibody reacts with the 57 kDa intermediate filament protein present in cells of mesenchymal origin [11].

The endometrial connective tissue was shown to contain vimentin intermediate filaments, whereas the invasive trophoblast showed no vimentin expression.

During the last decade the use of antibodies has been developed for both research and diagnostic purposes. However, in some cases there is a demand for detection of more than one antigen in a single tissue specimen. For a proper identification of co-localization and possible cell-to-cell spatial contacts, reliable double immunostaining is needed.

Conclusion

In this study, we demonstrated that cytokeratin and vimetin immunostains could be a standard for detection IT cells in the uterine curretage tissue.

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Uticaj aerozagađenja na učestalost hospitalizacije dece sa respiratornim oboljenjima

Impact of air pollution on the rate of hospital admission of children with respiratory diseases

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Apstrakt

Uvod/Cilj. Mnogobrojne epidemiološke studije pokazuju da aerozagađenje utiče na pojavu respiratornih oboljenja kod dece. U našoj zemlji do sada nije praćen uticaj aerozagađenja na hospitalizaciju dece zbog respiratornih oboljenja. Cilj ovog rada bio je da se prikaže kako tipični polutanti iz vazduha, sumpor dioksid i čađ, u koncentracijama koje se beleže u okviru redovnog monitoringa, utiču na hospitalizaciju dece zbog respiratornih oboljenja. **Metode.** Upoređivani su dnevni podaci o prosečnim koncentracijama sumpor dioksida i čađi u vazduhu sa dnevnim podacima hospitalizacije zbog respiratornih oboljenja kod dece uzrasta 0–14 godina u dva perioda (1992–1995. i 2002–2005. god.) u Nišu, Republika Srbija. **Rezultati.** U prvom posmatranom periodu bilo je ukupno 4 283 hospitalizacije dece zbog respiratornih oboljenja, dok je u drugom periodu bilo 3 842 hospitalizacije. U oba perioda najveći broj hospitalizacija utvrđen je kod dece uzrasta 0–4 godine, a najmanje kod dece uzrasta 10–14 godina. Statistički značajan uticaj polutanata na broj hospitalnih prijema zbog respiratornih oboljenja potvrđen je u periodu od 1992. do 1995. godine kod dece od 0–4 godine. Svako povećanje prosečne dnevne koncentracije čađi u vazduhu za 10 μg/m^3 dovelo je do povećanja broja hospitalizacija posle tri dana za 3,95% (CI 1,29–6,67%), posle četiri dana za 4,50% (1,77–7,30%), a posle sedam dana za 7,15% (1,21–13,44%). Povećanje prosečne dnevne koncentracije sumpor dioksida u vazduhu za 10 μg/m^3 dovelo je do povećanja broja hospitalnih prijema posle tri dana za 1,29% (0,03–2,56%). Uticaj aerozagađenja na broj hospitalnih prijema u starijim grupama, kao i u periodu od 2002. do 2005. godine nije bio statistički značajan. **Zaključak.** Koncentracije ispitivanih polutanata zabeležene u okviru redovnog monitoringa, van epizoda zagađenja, dovode do povećane hospitalizacije zbog respiratornih oboljenja kod dece uzrasta od 0 do 4 godine.

Ključne reči:
respiratorni trakt, bolesti; deca; vazduh, zagađenje; sumpor dioksid; prašina, hospitalizacija.

Abstract

Background/Aim. Numerous epidemiological studies have reported effects of air pollution on the prevalence of respiratory diseases in children. Association between air pollution and hospital admissions for respiratory diseases among children has not been investigated in our country yet. The purpose of this study was to examine impact of ordinary air pollutants (sulfur dioxide and black smoke) in concentrations regulary reported during monitoring on hospital admissions for respiratory diseases among children. **Methods.** We compared daily data of sulphur dioxide and black smoke concentrations in air with data of daily hospital admissions for respiratory diseases in children 0–14 years of age in two periods (1992–1995 and 2002–2005) in Niš, Serbia. **Results.** There were totally 4 283 and 3 842 hospital admissions for respiratory diseases in children in the first (1992–1995), and the second (2002–2005) period observed, respectively. The highest number of hospital admissions was registered in children aged 0–4 years, and the lowest one in children aged 10–14 years. Statistically significant influence of pollutants on the number of hospital admissions for respiratory diseases was observed in the period 1992–1995 in children aged 0–4 years. Overall, a 10 μg/m^3 increase in black smoke concentration was associated with a 3.95% (95% CI 1.29–6.67%) increase in the rate of hospital admission for respiratory diseases after three days, 4.50% (1.77–7.30%) after four days and 7.15% (1.21–13.44%) after seven days. A 10 μg/m^3 increase in sulphur dioxide concentration was associated with a 1.29% (0.03–2.56%) increases in the rate of hospital admission for respiratory illness after three days. Influence of air pollution on the number of hospital admissions in older groups of children, as well as in the period 2002-2005 was not statistically significant. **Conclusion.** Our study suggested that air pollution concentration measured during regular monitoring, out of episodes of pollution, appear to be risk for hospital admissions for respiratory diseases in children age 0–4 years.

Key words:
respiratory tract diseases; child; air pollution; sulfur dioxide; dust; hospitalization.

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Uvod

U zemljama u razvoju infekcije donjih partija respiratornog trakta glavni su uzrok morbiditeta i mortaliteta u dečijem uzrastu, dok u zemljama sa relativno dobro razvijenim zdravstvenim sistemom, kao što je naša, redak je smrtni ishod, ali respiratorne bolesti dosta opterećuju sistem zdravstvene zaštite [1]. Zbog toga je prevencija ovih bolesti veoma važna, kako za buduće zdravlje dece, tako i za smanjenje troškova zdravstvene zaštite [2].

Ispitivanja rađena u svetu utvrdila su da zagađenje vazduha u gradskoj sredini značajno utiče na zdravlje dece, posebno na respiratorni trakt [3–7]. U literaturi je najčešće praćen uticaj pojedinih polutanata iz vazduha na pojavu respiratornih simptoma i oboljenja, smanjenje plućnih funkcija, povećanu hospitalizaciju ili porast mortaliteta čak i kod koncentracija polutanata u vazduhu ispod preporuka Svetske zdravstvene organizacije [8].

U odnosu na odrasle, deca su osetljivija na delovanje aerozagađenja jer na kilogram telesne mase udišu mnogo veću količinu vazduha, pa samim tim unose u organizam i veću količinu polutanata, imaju uže vazdušne puteve i više vremena provode u raznim aktivnostima u spoljnoj sredini i to u vreme kada je aerozagađenje najveće [9, 10].

U našoj zemlji do sada nije praćen uticaj aerozagađenja na hospitalizaciju dece, te je cilj ovoga rada bio da se na primeru grada Niša pokaže kako tipični polutanti iz vazduha, sumpor dioksid i čađ, utiču na hospitalizaciju dece zbog respiratornih oboljenja.

Metode

Prezentovani podaci bazirani su na dnevnim informacijama o prosečnoj koncentraciji polutanata (sumpor dioksida i čađi) i hospitalizaciji zbog respiratornih oboljenja kod dece uzrasta 0–14 godina na teritoriji grada Niša. Ispitivanja su vršena u dva četvorogodišnja perioda (1992–1995. i 2002–2005. god.) koja su se međusobno razlikovala po nivou aerozagađenja u gradskoj sredini.

Podaci o broju hospitalnih prijema dece obolele od svih respiratornih bolesti (ICD J00–J99) po danima ispitivanih perioda dobijeni su od Centra za biostatistiku i informatiku Instituta za javno zdravlje Niš. Analizirani su posebno po starosnim grupama za decu od 0–4, 5–9 i 10–14 godina.

Korišćeni su podaci o aerozagađenju Centra za higijenu i zaštitu životne sredine Instituta za javno zdravlje Niš. Analizirane su prosečne dnevne koncentracije čađi i sumpor dioksida sa dve merne stanice na kojima su merenja vršena u svih osam godina po „Programu kontrole kvaliteta vazduha na teritoriji Republike Srbije“ za Ministarstvo zaštite životne sredine Republike Srbije.

Prosečne dnevne vrednosti za temperaturu, vazdušni pritisak i relativnu vlažnost vazduha za isti period dobijene su od Republičkog hidrometeorološkog zavoda Republike Srbije.

U statističkoj analizi primenjen je generalizovani linearni model (GLM) prema Poizonovom tipu regresije sa opcijom koja dozvoljava prekomernu disperziju vrednosti. U modelu je broj hospitalnih prijema definisan kao zavisno promenljiva, a koncentracija zagađujuće materije kao nezavisno promenljiva. Izvršeno je prilagođavanje modela u odnosu na kalendarsko vreme, temperaturu, relativnu vlažnost vazduha, vazdušni pritisak, dane u nedelji i sezonu (zimska-letnja). Prilagođavanje modela bilo je bazirano na Akaike informacionom kriterijumu (AIC) [11]. Korišćeni su oni periodi odloženog dejstva za meteorološke faktore koji su dali najniže vrednosti AIC. Na isti način bili su određeni stepeni slobode za funkcije regresionih kriva kojima je aproksimiran uticaj kalendarskog vremena i meteoroloških faktora. Dani u nedelji i sezona bili su uključeni u model kao kontrastne promenljive. Uticaj polutanta je aproksimiran pravom linijom. Analizirani su pojedinačni periodi odloženog dejstva polutanata 0–7 dana. Izračunate su vrednosti aproksimativnog relativnog rizika (OR – *odds ratio*) i 95% intervali poverenja (95% CI) za povećanje rizika od hospitalizacije zbog respiratornih oboljenja kod dece usled povišenja koncentracije polutanta za 10 μg/m^3. Analize su vršene korišćenjem statističkog paketa R u verziji 2.7.0 [12]. Specifična formulacija konačnog modela koji je korišćen bila je sledeća:

broj hospitalizacija = a + pkk (dan vremenske serije, kt = 32) + kontrast (sezona) + kontrast (dan u nedelji) + pkk (temperatura$_{lag = 0–3}$, kt = 4) + pkk (vlažnost vazduha$_{lag = 0}$, kt = 2) + pkk (vazdušni pritisak$_{lag = 0}$, kt = 3) + polutant$_{lag = 0}$

gde su: a – regresiona konstanta, pkk – prirodna kubna kriva sa određenim brojem kontrolnih tačaka (kt) i lag – period odloženog dejstva.

Rezultati

Za ispitivanje uzeta su dva četvorogodišnja perioda različita po koncentaciji sumpor dioksida i čađi u vazduhu (tabela 1).

U prvom periodu prosečne godišnje koncentracije sumpor dioksida dve godine bile su iznad GVI (granične vrednosti imisije) po našem nacionalnom standardu i to 1992. i 1993. god. [13]. U drugom periodu ispitivanja prosečne godišnje koncentracije sumpor dioksida bile su višestruko manje sa vrednostima i do tri puta nižim od GVI koja iznosi 50

Tabela 1

Prosečne godišnje koncentracije polutanata ($\bar{x}$±SD) u periodima 1992–1995. i 2002–2005. godine

Polutant (μg/m^3)	Period ispitivanja							
	I period (1992–1995)				II period (2002–2005)			
	1992.	1993.	1994.	1995.	2002.	2003.	2004.	2005.
Sumpor dioksid	65,6±74,1	67,1±73,8	32,3±47,3	35,5±31,7	15,6±10,8	17,7±16,1	13,6±12,2	14,9±13,9
Čađ	21,3±30,2	22,5±30,1	20,5±27,9	20,7±25,0	21,3±21,1	24,4±22,5	22,5±23,7	23,5±21,8

μg/m^3. Tokom oba perioda ispitivanja prosečne godišnje koncentracije čađi bile su značajno niže od GVI koja i za čađ iznosi 50 μg/m^3 na godišnjem nivou.

U periodu 1992–1995. god. prosečne godišnje koncentracije čađi iznosile su 21,3–22,5 μg/m^3, a sumpor dioksida 32,3–67,1 μg/m^3. Mann-Whitney *U* test pokazao je da su prosečne vrednosti koncentracija sumpor dioksida u periodu od 1992. do 1995. godine bile statistički značajno više nego u periodu od 2002. do 2005. godine ($Z = 24,63$ i $p < 0,001$), dok su prosečne koncentracije čađi bile statistički značajno više u periodu 2002–2005. god. ($Z = 10,30$ i $p < 0,001$). Povremeno, merile su se i jako visoke koncentracije ovih polutanata, te je tako maksimalno zabeležena prosečna dnevna vrednost sumpor dioksida u prvom periodu ispitivanja iznosila 434 μg/m^3, a u drugom 107 μg/m^3. U prvom periodu ispitivanja maksimalno zabeležena prosečna dnevna koncentracija čađi iznosila je 238 μg/m^3, a u drugom periodu 225 μg/m^3 (tabela 2).

zultovalo je porastom broja hospitalnih prijema posle tri dana od 1,29% (0,03–2,56%). Uticaj aerozagađenja na broj hospitalnih prijema u starijim grupama, kao i u periodu od 2002. do 2005. godine nije bio statistički značajan.

Diskusija

Uticaj povećanja koncentracije polutanata u vazduhu na ukupnu hospitalizaciju zbog respiratornih oboljenja zabeležen je u mnogim studijama, ali su se one uglavnom bavile ispitivanjem porasta broja hospitalnih prijema u toku epizoda zagađenja vazduha, kada su koncentracije polutanata bile izuzetno visoke, i do deset puta veće od preporučenih vrednosti [4–8, 14–19]. Ispitivanje rađeno u Engleskoj pokazalo je da je prijem u bolnicu za sva respiratorna oboljenja znatno veći u nedeljama sa epizodama aerozagađenja u odnosu na kontrolne nedelje [20].

Tabela 2

Prosečne dnevne vrednosti koncentracija polutanata, meteoroloških faktora i broja hospitalnih prijema dece u periodima 1992–1995. i 2002–2005. godine

Period ispitivanja	Praćeni parametri	x̄ ±SD	Med	Min–Max	Ukupno dece
1992–1995.	čađ (μg/m^3)	21,3±22,3	15,0	0,0–238,0	
	sumpor dioksid (μg/m^3)	50,9±48,6*	35,5	0,0–434,0	
	temperatura (°C)	12,1±9,0	12,8	13,2–31,2	
	relativna vlažnost (%)	67,5±13,7	68,0	30,0–96,0	
	vazdušni pritisak (mBar)	994,2±7,1	993,4	971,2–1018,8	
	hospitalni prijemi 0–4 g. (n)	1,36±1,34	1	0—7	1993
	hospitalni prijemi 5–9 g. (n)	1,08±1,32	1	0–7	1577
	hospitalni prijemi 10–14 g. (n)	0,49±0,79	0	0–6	713
					4283
2002–2005.	čađ (μg/m^3)	22,9±22,3*	16,5	0,0–225,0	
	sumpor dioksid (μg/m^3)	15,4±13,5	12,0	0,0–107,0	
	temperatura (°C)	12,1±8,9	12,7	11,6–30,4	
	relativna vlažnost (%)	70,5±13,1	72,0	26,0–98,0	
	vazdušni pritisak (mBar)	994,0±6,7	993,7	966,2–1014,8	
	hospitalni prijemi 0–4 g. (n)	1,44±1,42	1	0–10	2099
	hospitalni prijemi 5–9 g. (n)	0,75±1,05	0	0–10	1103
	hospitalni prijemi 10–14 g. (n)	0,44±0,82	0	0–6	640
					3842

*$p < 0,001$ prema odgovarajućoj vrednosti u poredbenoj grupi

U prvom posmatranom periodu bilo je ukupno 4 283 hospitalizacije dece zbog respiratornih oboljenja, dok je u drugom periodu bilo 3 842 hospitalizacije. U oba perioda najveći broj hospitalizacija utvrđen je kod dece uzrasta 0–4 godine (1 993 odnosno 2 099), a najmanje kod dece uzrasta 10–14 godina (713 odnosno 640).

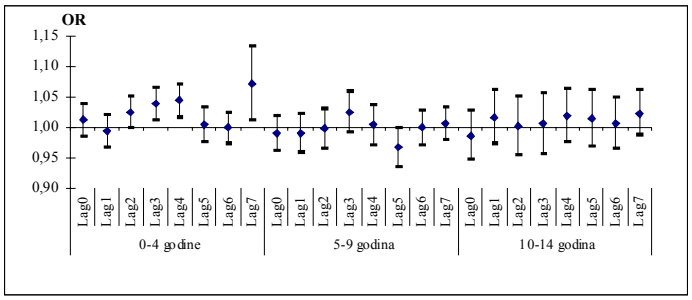
Uticaj povećanja koncentracija sumpor dioksida i čađi za 10 μg/m^3 na broj dnevnih hospitalizacija isti dan (lag 0) i za odloženi period do 7 dana posle izloženosti (lag 1 do lag 7) prikazan je na slici 1.

Statistički značajan uticaj polutanata na broj hospitalnih prijema zbog respiratornih oboljenja potvrđen je u periodu od 1992. do 1995. godine kod dece od 0–4 godine. Svako povećanje koncentracije čađi u vazduhu za 10 μg/m^3 dovelo je do povećanja broja hospitalnih prijema posle tri dana za 3,95% (1,29–6,67%), posle četiri dana za 4,50% (1,77–7,30%), a posle sedam dana za 7,15% (1,21–13,44%). Povećanje koncentracije sumpor dioksida za 10 μg/m^3 re-

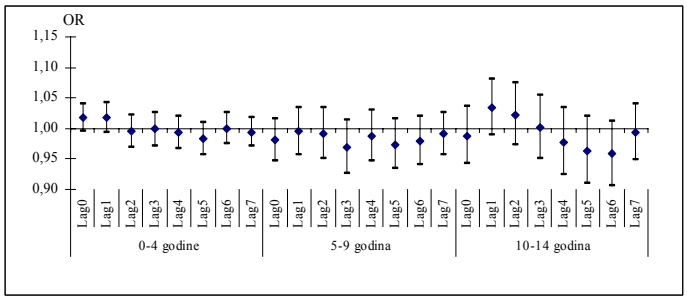
Najčešće je u literaturi praćena hospitalizacija celokupne populacije, jer je broj dnevnih hospitalnih prijema vrlo dobar pokazatelj akutnog efekta kratkotrajnih varijacija aerozagađenja [10]. Sve ove studije u manjoj ili većoj meri dokazale su uticaj ovako visokih koncentracija na porast hospitalizacije, posebno kod hroničnih bolesnika (astmatičara, bolesnika sa postojećim oboljenjima respiratornog i kardiovaskularnog sistema) i starijih osoba.

U literaturi ima jako malo podataka o uticaju aerozagađenja na hospitalizaciju dece. Ispitivani efekti aerozagađenja i polutanti razlikuju se u istraživanjima. Tako, od polutanata najčešće se prati uticaj čestica merenih kao PM_{10} ili $PM_{2,5}$, ozona i azotnih oksida. Istraživanja se uglavnom bave decom starosti do 5 godina ili decom obolelom od astme [21]. U našoj zemlji do sada nisu vršena slična ispitivanja.

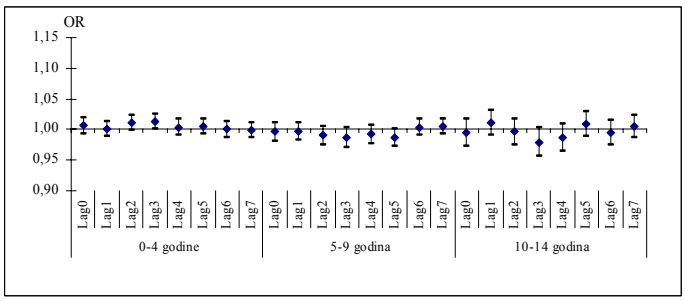
Studija rađena u Rimu pokazala je da sa povećanjem koncentracija azotnih oksida, ugljen monoksida i ozona u vazduhu raste i hospitalizacija dece zbog respiratornih oboljenja [22]. Do sličnih rezultata došle su i druge studije [23–27].



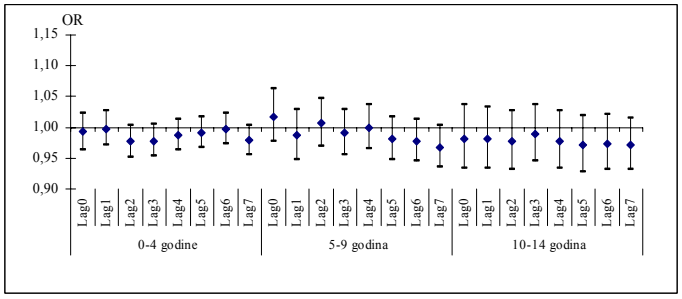
1 A. Čađ u periodu 1992–1995. god.



1 B. Čađ u periodu 2002–2005. god.



1 C. Sumpor dioksid u periodu 1992–1995. god.



1 D. Sumpor dioksid u periodu 2002–2005. god.

Sl. 1 – OR (95% CI) za uticaj povećanja koncentracija polutanata u vazduhu za 10 μg/m^3 na broj dnevnih hospitalnih prijema kod dece uzrasta 0–14 godina

Najnovija metaanaliza tri evropske studije koje su pratile hospitalizaciju dece starosti 0–14 godina pokazala je da porast čestica za 10 μg/m^3 dovodi do porasta hospitalizacije za 1%, a kod dece starosti 5–14 godina za 1,9% [28]. U našem istraživanju porast koncentracije čađi za 10 μg/m^3 doveo je do porasta hospitalizacija za 3,95% (posle tri dana), 4,50% (posle čatiri dana) i 7,15% (posle sedam dana), ali samo kod dece starosti 0–4 godine, koja su najmlađa i najosetljivija.

Većina do sada rađenih studija jasno ukazuje na povezanost visokih koncentracija ozona i čestica (PM_{10}) i broja hospitalnih prijema osoba obolelih od astme, kako odraslih, tako i dece [29–35]. Smatra se da je broj onih kod kojih se ispoljavaju efekti porasta koncentracija polutanata u vazduhu veći od prikazanog jer se samo najteži slučajevi hospitalizuju, dok većina bolesnika ne dospe do bolnice i završi u hitnoj službi ili kod pedijatra u ambulanti ili u domu zdravlja. Istraživanja su pokazala da od polutanata najčešće prisutnih u va-

zduhu gradske sredine, pored čestica, na povećanu hospitalizaciju utiču i ozon, sumpor dioksid i azotni oksidi [36–39].

Vrlo je teško u mešavini polutanata koja se nalazi u vazduhu odrediti koji je polutant najjače povezan sa zdravstvenim efektima, jer pokušaji da se izdvoje pojedini polutanti nisu dali zadovoljavajuće rezultate [40, 41].

Naše istraživanje pokazalo je da i koncentracije osnovnih polutanata zabeležene u okviru redovnog monitoringa, van epizoda zagađenja, dovode do povećanja hospitalizacije kod dece. Utvrđeno je da svako povećanje koncentracije ispitivanih polutanata za 10 $\mu g/m^3$ dovodi do povećanja hospitalnih prijema kod najmlađe dece posle tri, četiri i sedam dana (čađ), odnosno posle tri dana (sumpor dioksida). Na osnovu dobijenih podataka može se zaključiti da od dva ispitivana polutanta čađ ima značajniji uticaj na hospitalizaciju.

Da bi se dobili potpuniji podaci o ukupnom uticaju aerozagađenja, neophodno je ovim podacima dodati i podatke o dnevnim posetama lekaru zbog respiratornih oboljenja i broju intervencija službe hitne pomoći, ali se ovi podaci za sada u našoj zemlji ne prate kao obavezni. Takođe, treba dodati podatke iz privatnih bolnica i ambulanti koje rade na području grada.

Ne treba zanemariti ni činjenicu da je prvi period ispitivanja bio u vreme ekonomskih sankcija kada su i uslovi života građana bili znatno lošiji od perioda 2002–2005. god., te su na pojavu respiratornih oboljenja i povećanu hospitalizaciju sigurno uticali i mnogi socioekonomski faktori.

Ovo istraživanje, prvo ovog tipa na teritoriji naše zemlje, potvrdilo je da promene koncentracija polutanata i van epizoda zagađenja utiču na povećanje hospitalnih prijema zbog respiratornih oboljenja kod dece i da treba nastaviti sa ovim istraživanjima da bi se dobijeni rezultati potvrdili i utvrdili i kod ostalog dela populacije, posebno kod hroničnih bolesnika i starijih osoba. Praćenjem uticaja koncentracija polutanata koje su normalno prisutne u vazduhu naših gradova na hospitalizaciju i mortalitet može se doći i do zaključaka koji bi pomogli u reviziji postojećeg pravilnika i smanjenja GVI do nivoa koji ne utiče na zdravlje rizične populacije.

Zaključak

Koncentracije ispitivanih polutanata zabeležene u okviru redovnog monitoringa, van epizoda zagađenja, dovode do povećane hospitalizacije zbog respiratornih oboljenja kod dece uzrasta od 0 do 4 godine.

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Histopatološke karakteristike i koekspresija p53 i p16^{INK4a} proteina kod karcinoma bubrega

Histopathological characteristics and coexpression of p53 and p16^{INK4a} proteins in renal cancer

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Apstrakt

Uvod/Cilj. Renalni karcinomi (RK) čine histološki heterogenu grupu malignih tumora sa različitom kliničkom agresivnošću. Zastupljenost mutacije p53 u primarnom RK je retka, mada postoje podaci o njegovoj heterogenoj akumulaciji. Gubitak ekspresije proteina p16 u primarnom karcinomu bubrega detektuje se kod 20–30% bolesnika. Cilj ovog rada bio je da se utvrdi učestalost ispoljavanja mutiranog proteina p53 i ekspresija proteina p16^{ink4a} kod RK, analizira njihov međusobni odnos i odnos sa ispitivanim kliničkopatološkim parametrima. **Metode.** Ispitivanjem je bilo obuhvaćeno 12 bolesnika (66,7% muškaraca, 33,3% žena) sa patohistološki verifikovanim RK. Ekspresija mutirane forme proteina p53 i proteina p16 određivana je u tkivnim uzorcima, imunohistohemijskom analizom korišćenjem mišjih monoklonskih antitela firme DAKO, Denmark. **Rezultati.** Mutirani protein p53 utvrđen je kod 9 (75%) bolesnika, od kojih 66,6% istovremeno je imalo viši histološki gradus tumora (G3–4) i viši patološki stadijum bolesti (pT3a-b). Kod sedam (58,3%) bolesnika utvrđena je ekspresija proteina p16, dok je gubitak ekspresije proteina p16 utvrđen kod pet (41,7%) bolesnika. Utvrđena je statistički značajna pozitivna korelacija između patološkog stadijuma bolesti (TNM) i stepena diferentovanosti tumora (G), ($\rho = 0{,}834$ i $p < 0{,}001$), kao i između TNM i mitotskog indeksa ($\rho = 0{,}622$ i $p = 0{,}031$). **Zaključak.** Mutirana forma proteina p53 postojala je kod 75% bolesnika sa karcinomom bubrega, a 66,6% ovih bolesnika istovremeno imalo je i viši histološki gradus tumora i viši stadijum tumorske bolesti. Koekspresija mutiranog proteina p53 i proteina p16^{INK4a} kod karcinoma bubrega nije statistički značajna i nije u korelaciji sa kliničkopatološkim parametrima. Imunohistohemijska analiza mutiranog proteina p53 i ekspresija proteina p16 u karcinomu bubrega može imati prediktivni značaj.

Ključne reči:
karcinom renalnih ćelija; tumor supresorski protein p53; ciklin zavisni inhibitor kinaze p16; imunohistohemija.

Abstract

Background/Aim. Renal carcinoma represents histologically heterogeneous group of malignant tumors, with various clinical aggressiveness. The frequency of p53 mutation in primal renal carcinoma is rare, although there are information about its heterogeneous accumulation. The loss of protein p16 expression in primal renal carcinoma is detected in 20–30% of the cases. The aim of this paper was to determine frequency of mutated protein p53 and expression of protein p16^{INK4a} in renal carcinoma, to analyze their correlative relation and relation with the examined clinicopathological parameters. **Methods.** The examination included 12 patients (66.7% men, 33.3% women), with patohistologically verified renal carcinoma. Expression of mutated form of protein p53 and protein p16 was determined in tissue samples, by immunohistochemical analysis using of mice monoclonical antibodies produced by DAKO, Denmark. **Results.** In 9 (75%) of the cases was detected mutated protein p53, of whom 66.6% had higher histological gradus of tumor (G3-4) and higher pathological stadium of the disease (pT3a-b) at the same time. In 7 (58.3%) and 5 (41.7%) of the cases expression of protein p16, the loss of expression of protein p16 were detected respectively. A statistically significant positive correlation was determined between pathological stadium of disease (TNM) and the degree of tumor differentiation (G) ($\rho = 0.834$; $p < 0.001$), as well as between TNM and mitotic index ($\rho = 0.622$; $p = 0.031$). **Conclusion.** A mutated form of protein p53 exists in 75% of the cases with the renal carcinoma and 66.6% of then have higher histological gradus of tumor and higher stadium of tumor disease at the same time. Coexpression of mutated protein p53 and protein p16^{INK4a} in renal carcinoma is not statistically significant and it is not in correlation with clinicopathological parameters. Immunohistochemical analysis of mutated protein p53 in renal carcinoma can have predictive significance.

Key words:
carcinoma, renal cells; tumor suppressor protein p53; cyclindependent kinase inhibitor p16; immunohistochemistry.

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Uvod

Renalni karcinomi (RK) su malignomi koji nastaju od epitela bubrežnih tubula i čine preko 90% svih malignih tumora bubrega odraslih osoba oba pola i 3% svih karcinoma. Najnovija histološka klasifikacija tumora bubrega Svetske zdavstvene organizacije (SZO) klasifikuje ove tumore u nekoliko kategorija od kojih je najčešći karcinom renalnih ćelija. Renalni karcinomi pokazuju različite histološke tipove ćelija ili se, pak, ovi tipovi međusobno kombinuju. Kod tumora bubrega analiziraju se tri osnovna morfološka parametra: citološki elementi – tip tumorskih ćelija, histološki tip: solidni, acinarni, tubulopapilarni, cistični i citološko gradiranje stepena maligniteta bazirano na stepenu jedarne atipije: G1–4 (najčešće se koristi sistem gradiranja po Fuhrmanu) [1]. Poslednjih decenija incidencija i mortalitet RK su u porastu [2].

Renalni karcinomi su heterogeni tumori koji uključuju različite entitete, od ranga dobre biološke prognoze do ranga ekstremno različitog i agresivnog toka. Heterogenost tumora utiče na preživljavanje i ishod bolesti, a njihova procena je veoma važna za prognozu bolesti. Najvažnija determinanta za ishod bolesti je anatomska ekstenzija tumora tj. patološki stadijum tumorske bolesti, dok u druge, ne manje značajne prognostičke morfološke faktore, spadaju histološki tip i nuklearni gradus tumora [3].

U procesu maligne transformacije epitelnih ćelija bubrežnog parenhima, pored drugih faktora, važnu ulogu imaju i promene u tumor supresorskim genima i ćelijskim protoonkogenima, odnosno promene u genima čiji proteini kontrolišu rast i deobu ćelija. U kontroli ćelijskog ciklusa direktnu ulogu imaju supresor geni p53 i $p16^{ink4a}$, kao i retinoblastom gen, tj. geni čiji su proteini uključeni u prenos komunikacijskih signala izvan ćelija do ćelijskog jedra. Ove promene se izučavaju kako zbog njihovog značaja u procesu kancerogeneze, tako i sve veće primene u dijagnostičke, prognostičke i terapeutske svrhe. Tumor supresorski gen p53 enkodira protein koji inhibiše nastajanje tumora tako što utiče na stabilnost genoma, rast i diferencijaciju ćelije i stimulaciju apoptoze. Međutim, mutirani tip gena p53 menja strukturu i funkciju enkodiranog proteina i inicira proces onkogeneze [4].

Supresorski gen $p16^{INK4a}$ je multipli tumor supresor gen koji inhibiše fosforilizaciju retinoblastom proteina i uzrokuje zastoj ćelijskog ciklusa u fazi G1. Mutirani $p16^{INK4a}$ gen je inaktiviran zbog čega ćelije nekontrolisano rastu i maligno se transformišu [5, 6].

Kliničke, radiološke i patohistološke karakteristike renalnog karcinoma daju značajne prognostičke informacije. Međutim, u mnogim slučajevima zbog heterogenosti tumora ovi parametri su insuficijentni te se uvode novi biomolekulski markeri i onkogeni kao potencijalni dijagnostički i prognostički parametri [7].

Cilj ovog rada bio je da se utvrdi učestalost mutiranog proteina p53 i ekspresije proteina $p16^{ink4a}$ u RK, analizira njihov međusobni odnos i korelacija sa ispitivanim kliničko-patološkim parametrima.

Metode

U radu je korišćen materijal dobijen u toku radikalnih nefrektomija (RN) urađenih u Hirurškom odeljenju Vojne bolnice u Nišu u periodu 2004–2006. godinr kod 12 bolesnika sa klinički lokalizovanim/lokalno uznapredovalim RKe. Dijagnostika RK učinjena je prema uobičajenom kliničkom protokolu koji je obuhvatao klinički pregled, pregled abdomena i karlice ultrazvukom, nativnu i kontrastnu radiografiju, renalnu arteriografiju i patohistološku verifikaciju. Preoperativno, bolesnicima nije ordinirana zračna terapija. U okviru RN kod svih bolesnika učinjena je adrenalektomija i klasična (neproširena) regionalna limfadenektomija. Patohistološkim izveštajem objedinjeni su standardni parametri prema protokolu za prijavljivanje RK nakon RN. Patohistološku dijagnozu RK kod svih bolesnika, odredili smo iz materijala dobijenog RN. Za analizu smo koristili metodu delimične obrade tkiva. Mikroskopski smo analizirali prosečno po 10 tkivnih isečaka uzetih iz tumora bubrega. Isečci tkiva fiksirani su u puferisanom 4% formalinu, 18–24 sata, dehidrisani u alkoholu i ukalupljivani u parafin. Iz parafinskih blokova tkivo je sečeno na debljinu od 5–7 μm, bojeno standardnom hematoksilin eozin metodom (H&E) i mikroskopski analizirano na mikroskopu Microstar H110 (Reichert-Jung, Buffalo, USA).

Određivan je histološki tip tumora, nuklearni gradus tumora po Fuhrmanu i patološki stadijum bolesti (pT).

Histološki gradus tumora određivan je na osnovu nuklearnog gradusa po Fuhrmanu i označavan je kao dobro diferentovan (G1), srednje diferentovan (G2), loše diferentovan (G3) i nediferentovan (G4).

Za utvrđivanje stadijuma tumorske bolesti (pT) koristili smo klinički stadijum određivan prema klasifikaciji *American Joint Committetee on Cancer – tumor, lymph node and distant metastases staging (*AJCC-TNM) iz 2005. godine.

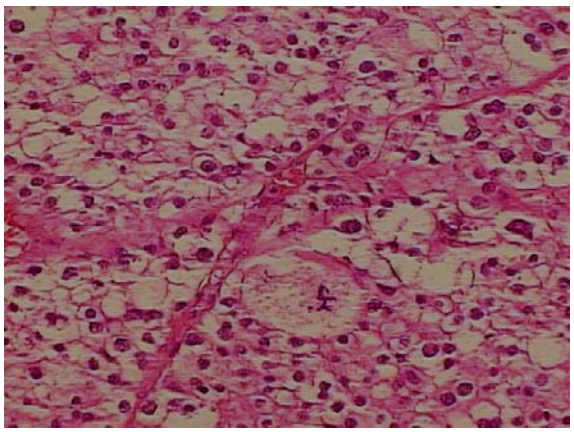
Mitotski indeks određivan je u područjima tumora sa najvećim brojem mitoza na 10 polja najvećeg mikroskopskog uveličanja, × 400 po uzorku (ukupan broj mitoza/10 *High Power Field* – HPF). Mitotski indeks utvrđen je na sledeći način: 0 – nema mitoza; nizak mitotski indeks/*low index*: 1–6 mitotskih figura/10 HPF; visok mitotski indeks/*High index*: > 6 mitotskih figura/10 HPF.

Na reprezentativnim presecima tumorskog tkiva primenjena je imunohistohemijska tehnika obeleženog streptavidin-biotina (*labelled streptavidin-biotin* – LSAB+ metoda) uz imunoperoksidazu prema odgovarajućoj proceduri. Korišćena su komercijalna primarna monoklonska mišja antitela (p53 i $p16^{INK4a}$ kompanije DakoCytomation, Glostrup, Danska) protiv p53 (klon DO-7, razblaženje 1 : 50) i $p16^{ink4a}$ (klon E6H4 histološki komplet). Kao sistem za vizuelizaciju korišćen je Dako En Vision $^{(TM)}$ komplet, kataloški broj K5007 i hromogen Dako Dab liquid, kataloški broj 3466. Preparati su kontrastirani Mayerovim hematoksilinom.

Imunohistohemijskom analizom utvrđen je intenzitet bojenja (1+ – slabo bojenje, 2+ – umereno bojenje, 3+ – intenzivno bojenje) i procenat pozitivnih tumorskih ćelija (0% nema imunoreaktivnih ćelija, 1–5% retke pozitivne ćelije, 6–25% malo pozitivnih ćelija, 26–75% mnogo pozitivnih ćeli-

ja, >75% većina pozitivnih ćelija). Imunoreaktivne tumorske ćelije p16 pokazivale su samo nuklearno svetlobraon prebojavanje.

Za opis karakteristika formiranih grupa ispitanika upotrebljeni su standardni deskriptivni statistički parametri: aritmetička sredina ($\bar{x}$), standardna devijacija (SD), amplituda varijacija (min–max), indeks strukture (%). Za testiranje normalnosti raspodele ispitivanih obeležja korišćen je Šapiro-Vilksov test (*Shapiro-Wilks*). Korelacija p53 alteracije i $p16^{INK4a}$ ekspresije sa starošću ispitanika, kao i sa patohistološkim parametrima, ispitivana je Spirmanovim koeficijentom korelacije rangova (*Spearman's rho* – ρ). Za unos podataka i statističku obradu korišćen je SPSS *for Windows* (Ver. 8.0).



Sl. 1 – Svetloćelijski karcinom bubrega, sa G3 Fuhrman gradusom (H&E, × 200)

Rezultati

Mikromorfološkom i imunohistohemijskom analizom obuhvaćeno je 12 bolesnika sa karcinomom bubrega Urološkog odeljenja Vojne bolnice u Nišu. Prosečna starost ispitivanih bolesnika bila je 66,8 ± 9,8 godina; najmlađi bolesnik je imao 53 godine, a najstariji 82 godine. U odnosu na pol bilo je 8 (66,7%) muškaraca i 4 (33,3%) žene.

Kod svih bolesnika dijagnostikovan je jednostrani karcinom bubrega (7 ili 58,3% u levom i 5 ili 41,7% u desnom bubregu). Nije nađena statistički značajna razlika u zastupljenosti karcinoma levog ili desnog bubrega između ispitanika različitog pola (*Fisher exact test*: $p = 0,575$; $p > 0,05$).

Najčešći histološki tip tumora bio je svetloćelijski, konvencionalni tip i nađen je kod 7 (58,4%) bolesnika (slika 1). Kod tri (25%) bolesnika nađen je papilarni histološki tip, a kod preostala dva slučaja utvrđeni su hromofobni karcinom i karcinom sabirnih kanala (*Collecting duct carcinoma* – Bellini).

Određivanjem međuzavisnosti između procenta pozitivnosti i intenziteta bojenja p53, dobijena je statistički značajna pozitivna korelacija ($\rho = 0,859$; $p < 0,001$). Statistički značajna korelacija, uočena je i između procenta pozitivnosti i intenziteta bojenja p16 ($\rho = 0,923$; $p < 0,001$).

Prema stepenu diferentovanosti, najviše je bilo srednje diferentovanih karcinoma (G2) 50%, potom 33,3% loše diferentovanih (G3), 16,7% nediferentovanih (G4), dok dobro diferentovanih karcinoma (G1) nije bilo.

Uočena je statistički značajna pozitivna korelacija između TNM i stepena diferentovanosti tumora (G), ($\rho = 0,834$; $p < 0,001$), kao i između TNM i mitotskog indeksa ($\rho = 0,622$; $p = 0,031$). Najveći broj bolesnika, 50%, bio je sa niskim mitotskim indeksom (1+), sa umerenim mitotskim indeksom (2+) bilo je 41,7% i sa visokim mitotskim indeksom (3+) 8,3% bolesnika.

Korelacija ekspresije proteina p53 i $p16^{INK4a}$ sa starošću i kliničkopatološkim parametrima nije bila statistički značajna (tabele 1 i 2).

Mutirani protein p53 verifikovan je kod devet (75%) bolesnika, dok kod tri (25%) bolesnika nije bilo ekspresije. U odnosu na intenzitet nuklearne ekspresije proteina p53, kod osam (67%) bolesnika utvrđen je slab i umeren intenzitet bojenja (slika 2), dok je samo kod jednog bolesnika nađeno intenzivno bojenje. Kod 66,6% bolesnika sa mutiranim proteinom p53 istovremeno utvrđen je viši histološki gradus tumora (G3-4) i viši patološki stadijum bolesti (pT3a-b).

Tabela 1

Unakrsne korelacije histopatoloških parametara

	Starost		Fuhrman (G)		Mitotski indeks	
Parametri	ρ	*p*	ρ	*p*	ρ	*p*
TNM	0,164	0,610	0,834	0,000	0,622	0,031
Mitotski indeks	0,560	0,058	0,501	0,097	/	/

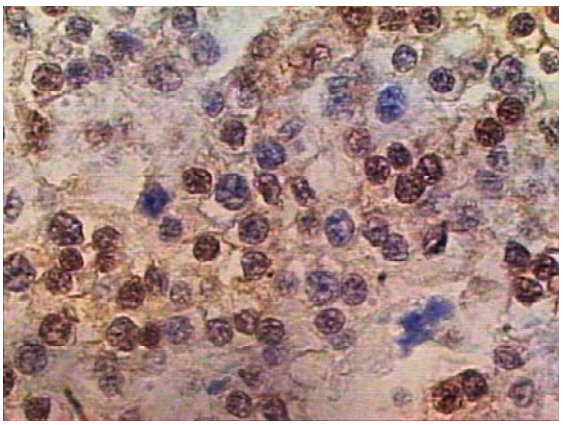
TNM – tumor, limfni čvor, metastaze klasifikacija; G – Nuklearni gradus tumor

Tabela 2

Korelacija ekspresija p53 i p16 sa starošću bolesnika i histopatološkim parametrima

Parametri	Procenat pozitivnosti		Intenzitet bojenja	
	ρ	*p*	ρ	*p*
Starost*	-0,373	0,232	-0,316	0,316
Fuhrman (G) *	-0,064	0,843	0,034	0,915
TNM*	-0,080	0,804	0,101	0,756
Mitotski indeks*	-0,082	0,800	0,134	0,678
Starost†	-0,166	0,605	-0,273	0,390
Fuhrman (G)†	0,255	0,424	0,242	0,448
TNM†	0,368	0,239	0,267	0,401
Mitotski indeks†	0,018	0,955	-0,139	0,668

* – korelacija ekspresije p53; † – korelacija p16 ekspresije; G – nuklearni gradus tumor



Sl. 2 – Fokalna umerena nuklearna imunoreaktivnost za p53 protein kod slabo diferentovanog karcinoma bubrega (L SAB, × 200)

Kod sedam (58,3%) bolesnika nađena je ekspresija proteina $p16^{INK4a}$ u tumorskim ćelijama, slabog do izraženog intenziteta, dok je gubitak ekspresije proteina p16 nađen kod pet (41,7%) bolesnika. Ukupno 71% bolesnika sa verifikovanim proteinom p16 istovremeno imalo je viši histološki gradus tumora (G3-4) i viši patološki stadijum bolesti (pT3a-b).

Istovremeno prisustvo mutiranog proteina p53 i ekspresija proteina $p16^{INK4a}$ kod karcinoma bubrega nije statistički značajno i nije u korelaciji sa kliničkopatološkim parametrima.

Diskusija

Renalni karcinom je skoro isključivo tumor odraslih i svake godine se dijagnostikuje oko 30 000 novih slučajeva u SAD. Njegova učestalost raste sa svakom dekadom života ispod 6. dekade i 2–3 puta je češći kod muškaraca. Retko se javlja u prve dve dekade života, uključujući samo 2% tumora bubrega kod dece [2].

Iako su gojaznost, pušenje i izlaganje industrijskim hemijskim jedinjenjima mogući faktori rizika u genezi RK, u većini slučajeva patogeneza je nejasna.

Klinički tok RK je nepredvidljiv, ali sa dobro dokumentovanim slučajevima spontane regresije metastaza [3].

Renalni karcinom ima jasne morfološke osobine i nastaje kroz konstelaciju raznih genetskih oštećenja. Otkrića u genskoj osnovi karcinoma ukazala su na činjenicu da je ova bolest delimično bolest gena. Na molekulskom nivou, karcinogeneza je rezultat akumulacije genskih lezija koje se dešavaju na onkogenima i tumorsupresorskim genima. Izmenjena funkcija ovih gena dovodi se u vezu sa procesima maligne transformacije ćelija [8].

Podaci iz literature pokazuju da su podtipovi karcinoma bubrega udruženi sa različitim genskim oštećenjima. Naime, različiti supresor geni su zahvaćeni u raznim formama karcinoma bubrega. Većina nepapilarnih svetloćelijskih karcinoma bubrega udružena je sa gubitkom tumor supresor gena na kratkom kraku 3. hromozoma [9].

Tumor supresorski gen p53 stimuliše transkripciju gena vezanih za zaustavljanje ćelijskog ciklusa ili programiranu ćelijsku smrt, apoptozu. Ukoliko postojeći regulatorni mehanizmi u ćeliji ne uspeju da reparišu DNK i očuvaju integritet genoma, gen p53 postaje pokretač programirane ćelijske smrti i onemogućava dalju reprodukciju izmenjene ćelije [4, 10, 11].

Mutacije gena p53 menjaju strukturu i funkciju enkodiranog proteina koji kao takav nije u mogućnosti da očuva integritet genoma, te strukturne i fizičke modifikacije molekula p53 iniciraju proces onkogeneze. Genske mutacije p53 opisane su kod preko 50% tumora ljudi, npr. kod sarkoma, leukemije, karcinoma kolona, pluća, mokraćne bešike i prostate. Visoka učestalost ovih mutacija u kanceru kod ljudi reflektuje važnost inaktivacije p53 za proces tumorogeneze [12]. Učestalost mutacija p53 u primarnim renalnim karcinomima retka je, mada postoje podaci i o heterogenoj akumulaciji [13]. Imunohistohemijskom identifikacijom utvrđeno je da nemutirani molekul proteina p53 ima kratak poluživot kod normalnih ćelija, dok disfunkcionalni kompleksi p53 pokazuju produženi poluživot i utiču na intranuklearnu akumulaciju proteina p53 koji se može detektovati korišćenjem konvencionalnih tehnika koje smo i mi primenili. U istraživanjima drugih autora pozitivno p53 imuno bojenje najčešće je udruženo sa visokim gradusom tumora i mitotičkim indeksom, kao i konvencionalnim markerima loše prognoze što pokazuje i naša studija [14].

Naši rezultati pokazuju da je ekspresija mutiranog proteina p53 česta pojava kod karcinoma bubrega sa višim histološkim gradusom tumora i višim stadijumom bolesti, što može ukazivati na progresiju tumorske bolesti.

Oštećenje tumor supresorskog gena $p16^{INK4a}$ prisutno je kod različitih humanih malignoma uključujući razne tipove gastričnog karcinoma, karcinom mokraćne bešike i pluća, maligni melanom i mnoge druge. Najčešći mehanizam njegove inaktivacije je tačkasta mutacija i delecija, ali se spominje i metilacija promoter oblasti [15]. Kod primarnih RK gubitak heterozigotnosti na 9p hromozomu otkriva se kod 20–30 % bolesnika, dok je inaktivacija gena $p16^{INK4a}$ homozigotnom delecijom ili tačkastom mutacijom retka [16].

Od kada je otkriven kao inhibitor ciklin zavisne kinaze 4 i 6, tumor supresor gen $p16^{INK4a}$ postao je atraktivan u otkrivanju karcinogeneze. Dosadašnje studije su pokazale da postoji značajna korelacija između smanjene ekspresije $p16^{INK4a}$ i tumorske progresije kod minimalno invazivnog urotelijalnog karcinoma [17].

Prema nekim autorima gubitak ekspresije proteina $p16^{INK4a}$ u primarnom karcinomu bubrega detektuje se kod 20–30% bolesnika, dok je u našoj studiji gubitak verifikovan čak kod 41,7% bolesnika, što takođe može ukazivati na njegovu moguću ulogu u progresiji renalnog karcinoma [18].

Zaključak

Rezultati naše studije pokazuju da mutirana forma proteina p53 postoji kod 75% bolesnika sa karcinomom bubrega. Češća je kod svetloćelijskog histološkog tipa i 66,6% ovih bolesnika istovremeno ima i viši histološki gradus tumora i viši stadijum tumorske bolesti.

Kod 58,3% bolesnika postoji ekspresija proteina $p16^{INK4a}$ u tumorskim ćelijama, dok gubitak ekspresije proteina $p16^{INK4a}$ postoji kod 41,7% bolesnika.

Imunohistohemijska analiza mutiranog proteina p53 i ekspresija proteina $p16^{INK4a}$ u karcinomu bubrega može imati prediktivni značaj, jer ukazuje na progresiju primarnog renalnog karcinoma.

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Effects of perineural steroid injections on median nerve conduction during the carpal tunnel release

Delovanje perineuralne primene kortikosteroidnih injekcija na sprovodljivost medijalnog nerva tokom oslobađanja karpalnog tunela

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Abstract

Background/Aim. The treatment outcome of the median nerve compressive neuropathy in the carpal zone due to carpal tunnel syndrome (CTS) is represented by recovering the nerves sensibility, conductivity, condition and strength. Perineural application of betamethasone during the surgical decompression might result in faster recovery of compressed median nerve's conduction speed. **Methods.** In this study 40 patients with CTS were randomly divided in the two groups. In the first group (n = 20) we performed the surgical decompression of the median nerve by the open release of the carpal tunnel, and in the second group (n = 20) we applicated a perineural injection of 1 ml of betamethason immediately after the surgical decompression. We performed the electrodiagnostic (ED) examinations 7, 30 and 90 days after the surgery, and measured the conduction speed of the median nerve in the carpal tunnel zone and the sensitivity conduction speed of the median nerve. **Results.** Significant differences in examined ED respective variable values in different time intervals were obtained. At the final measurements, 90 days after the surgical procedure, both groups evidenced a full recovery of the conduction speed in the carpal tunnel with statistically significant better results in the second group of the patients (t = -2.116; p = 0.043). **Conclusion.** Intraoperative application of the corticosteroid injection during the surgical decompression results in faster regaining of conduction speed of the median nerve.

Key words:
carpal tunnel syndrome; reconstructive surgical procedures; intraoperative period; betamethasone; injections; median nerve; electrodiagnosis.

Apstrakt

Uvod/Cilj. Ishod lečenja kompresivne neuropatije medijalnog nerva nastale kao posledica sindroma karpalnog tunela (SKT) manifestuje se oporavkom nervne osetljivosti, sprovodljivosti, funkcije i jačine. Cilj rada bio je da se ustanovi da li perineuralna primena betametazona tokom hirurške dekompresije može ubrzati oporavak sprovodljivosti medijalnog nerva. **Metode.** U ispitivanje je bilo uključeno 40 bolesnika sa SKT koji su metodom slučajnog izbora bili podeljeni u dve grupe. Dvadeset bolesnika prve grupe podvrgnuto je hirurškoj dekompresiji medijalnog nerva oslobađanjem karpalnog tunela. Dvadeset bolesnika iz druge grupe tokom iste hirurške intervencije dobilo je perineuralnu injekciju 1 ml betametazona neposredno nakon hirurške dekompresije. Elektrodijagnostička ispitivanja sprovedena su sedam, 30 i 90 dana nakon operacije. Merena je brzina provođenja medijalnog nerva u karpalnom kanalu i senzitivna brzina provođenja *n. medianus-a.* **Rezultati.** Elektrodijagnostička ispitivanja pokazala su statistički značajno bolje rezultate u odnosu na stanje pre hirurškog zahvata u sva tri vremenska intervala praćenja nakon operacije. Ispitivanja sprovedena nakon 90 dana od operacije kod obe grupe bolesnika pokazala su puni oporavak brzine sprovodljivosti u predelu karpalnog tunela, ali su statistički značajno bolji rezultati dobijeni u drugoj grupi bolesnika (t = -2,116; p = 0,043). **Zaključak.** Intraoperativno davanje injekcije kortikosteroida nakon hirurške dekompresije značajno ubrzava normalizaciju brzine sprovodljivosti medijalnog nerva.

Ključne reči:
karpusni tunel, sindrom; hirurgija, rekonstruktivna, procedure; intraoperativni period; betametazon; injekcije; n. medianus; elektrodijagnostika.

Introduction

In the past two decades a number of scientific studies were performed helping to understand the causes and process of developing the carpal tunnel syndrome (CTS), clinical forms of the disorder, and ways of treatment of the median nerve compressive neuropathy in the carpal zone [1]. Nonsurgical treatments, especially the corticosteroid injections, have given good results initially. Steroid injections provide calming of an inflammatory process and help to reduce swelling

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of the compressed nerve, thus, the therapy results in loss of symptoms and in recovery of electrodiagnostic (ED) parameters. In the early stages of the disease the outcome of the nonsurgical treatment is satisfactory. Long term assessment of patients with carpal tunnel syndrome (CTS) registered a number of recidives, so that the common standing point of contemporary medicine is that for the majority of CTS cases the only successful treatment is a surgical one. The later refers particularly on the mild and severe forms of the disorder, whereas the conservative treatment is reserved for the low severity compression neuropathy cases and for the patients who lack the motivation for the surgical intervention. The treatment today is aimed not only to resolve the symptoms of the disease, but to find a way to the fastest and best restitution of whole hand and compressed nerve functions.

Many physicians use their own protocol of examinations and clinical tests to make diagnosis [2]. Some autors disclaim the necessity of electrophysiologic examinations, while others consider them as extraordinary important [3–6]. During the history taking and physical examinations of the patients, it is not enough just to make the CTS diagnose, but it is necessary to estimate the stadium of the disorder, i.e. the severity of the compressive neuropathy [7].

Therefore, the treatment outcome of the median nerves compressive neuropathy in the carpal zone might differ considerably, depending on time of making an exact diagnosis of the disease, and on the choice of the treatment method. The outcome may be interpreted as subjective and objective one. Objective results of recovering the nerves sensibility, conductivity, condition and strength are those got from clinical and ED testing. The achieved recovery of the diseased hand is usually evaluated through data obtained by the functional tests, ED examinations, present complications, and a time needed to return to work [8–10]. Subjective results given by the patient himself, such as disappearing of symptoms and relief, usually are not referred in the outcome report of the treatment [11, 12].

Phalen's [13] conclusion in his report was that the relief of the symptoms produced by the steroid injections confirms the CTS diagnosis. Katz et al. [14] give in their studies the detailed description of positive effects provided by the steroid injections into the carpal tunnel. There are a lot of studies comparing the outcomes of steroid injection treatments with those obtained by surgical decompression [15, 16]. There is no doubt that a surgical treatment results in better and long-term outcomes. Although the surgical treatment usually is not followed by high risks, and is a common surgical procedure, it involves some discomfort, thus it is a common practice to proceed the surgery by a conservative treatment, having considered the severity of the compressive neuropathy. Edgell et al. [17] confirmed their hypothesis that the surgical treatment preceded by the steroid injections, resulted in better outcome.

Considering good, but short-term effects of the steroid injections, we supposed that the application of the steroid injection during the surgical procedure, might provide faster regain of compressed nerves conduction speed, and, consequently, faster recovery of the median nerve. The research on the outcomes of the intrasurgical application of steroid injection has been performed with great caution, considering that, to our best knowledge, none of the surgical methods up to now gave significantly better outcome than the others.

Methods

The study was performed on a group of 40 patients with clinically obvious CTS, treated at the Clinic for Plastic Surgery and Burns, Military Medical Academy in Belgrade, 2005 and 2006.

According to the up-to-date knowledge, recovery of the sensitive and motion functions in majority of surgically treated patients is obvious, but can take weeks or months. Aiming to shorten this time as much as possible, we decided to applicate 1 ml of betamethasone during the surgery, immediately after the decompression. For the evaluation of the applied treatment method, we used objective results of measurements concerning the recovery of median nerve sensibility and conductivity, performed with parameters of ED examinations. There were examinations of the combined median nerve conductive speed in the carpal tunnel zone, and sensitive conduction speed.

We divided the patients in the two groups. In the first group we performed the surgical decompression of the median nerve by the open release of the carpal tunnel, and in the second group we applicated a perineural injection of 1 ml of betamethasone immediately after the surgical decompression.

Aches, "pins and needles", stiffness and limited motion of the diseased hands were registered in all patients before the treatment. The following provocative tests were performed in each patient: Phalen's test, Tinel's test and Durkan's test for median nerve compression.

Together with clinical tests, the neurophysiologic examination of characteristics of the median nerve (EMNG) was performed in all the examinees. Measurements of the combined median nerve conduction speed in the carpal tunnel zone, and of the sensitive speed of median nerve conduction, were evaluated.

To avoid problems of statistical nonindependence in the analysis, only one operation from each patient was used, even from those who had CTS on both hands.

After the presurgical treatment and preparation, each patient was operated on either in regional intravenous, or in axillary block anesthesia using the tourniquet. The same surgery technique of the open carpal tunnel release for decompressing the nerve was performed in each patient.

The incision was set in the projected axis of the fourth finger, beginning at the middle part of the palm, following the proximal part between the thenar eminences, till the proximal wrist crease. After the incision of the skin and subcutaneous tissue was made, deep structures were identificated by a careful dissection. Eye-controlled directly, the transversal carpal ligament was cut, to proceed to the release of the median nerve from its surrounding structures. In the cases where the surrounding tissue was thickened, it was extirpated. The epineurectomy was not performed, since a plenty of studies have shown that the procedure did not brought any difference in better recovering of the com-

pressed nerve. In the course of the nerve preparation, mandatorily the motion branch was identified and released. After the thorough hemostasis, the wound was sutured in one layer. In 20 patients, after the decompression of the nerve, and after the partial suture of the surgical wound, 1 ml betamethasone was injected perineuraly trough a plastic catheter. After the application of the corticosteroid, the plastic catheter was pulled out and wound suture completed. In all patients, hands were bandaged up without a splint. The patients were told that they could start to slightly move their fingers right after the operation.

In all the patients, a controlled physical therapy started up on the first postoperative day. Daily, after removing the bandages, a therapy with "Bioptron" lamp was performed, together with the laser therapy. A laser tube of 820 nm and a 1000 Hz frequency was used. After the physical therapy, hands were again bandaged up, until the next day therapy. On the tenth day after the surgical operation, kinesitherapy and electrophoresis started up, with the application of potassium iodide and novocain. Equal physical therapy during twenty days was applied in all patients.

Seven, thirty and ninety days after the treatment, neurophysiologic examinations of median nerve were repeated in examinees, performed by the same examiner and with use of the same equipment, always followed by the analysis of the characteristics of the treated nerve. Clinical tests were repeated as well, and it was registered either presence or absence of symptoms that were noticed before the treatment.

The outcome of the applied treatment method was assessed by the patient's subjective feelings, provocative clinical tests, and results of ED examinations.

Results

Of 40 patients, 27 (67.5%) were women and 13 (32.5%) were men. Their age ranged from 29 to 80 years with a mean of 51.6 years.

In examined patients, a disappearing of before-surgery symptoms after treatments applied was promminent as seen in the table 1. In three quarters of patients, we registered that all symptoms had been present before the surgery dissapeared within only seven days after the surgical procedure. Disappearing of symptoms was almost equal in both groups of patients, and final measurements showed that in only two patients of the first group and in one of the second group, presence of some previous symptoms was registered (Table 1).

Table 1

Disappearing of the symptoms of the median nerve compressive neuropathy

Treatment method	Days after surgery		
	7	30	90
Surgery	75%	90%	95%
Surgery and betamethason intraoperatively	77.5%	90%	97.5%

Note: the results are given as percentage of patients without the simptoms

Mean values of the combined median nerve conduction speed (CS) in the carpal tunnel zone, measured before the surgical treatment, were almost identical in both first and second group of patients (CS1 = 25.487 msec, and CS2 = 25.387 msec, respectively). The values largely overpas the reference values of 45 msec. First measurements, performed seven days after the surgical operation, did not show a significant recovery of the conduction speed in neither group of patients, although a slight recovery, attending the respective values of CS1 = 27.407 msec and CS2 = 29.420 msec, were registered. Measurements performed in the second term, after one month, showed that in both groups there were enhanced improvements of the conduction speed, particularly in the second group where 1 ml of betamethasone was given to each patient in the course of the surgical operation, although in neither group the reference values were attended yet. In the second group of the patients statistically significant better recovery of the nerve function was achieved (t = -2.365; p = 0.025). At the final measurements, 90 days after the surgical operation, both groups evidenced a full recovery of the conduction speed in the carpal tunnel with statistically significant better results in the second group of the patients (t = -2.116; p = 0.043). On the other words, statistically significant faster and better recovery of the conduction speed in the carpal tunnel was achieved in the patients treated with the application of betamethasone during the surgical procedure (Table 2).

Table 2

Pre- and postoperative values of the median nerve conduction speed

Treatment method	Examined variable (msec)	Before surgery	Days after surgery		
			7	30	90
Surgery	conduction speed	25.487	27.407	36.027	47.027
	sensitive conduction speed	25.387	29.420	41.093*	50.147*
Surgery and beclomethasone intraoperatively	conduction speed	32.200	32.727	38.160	45.347
	sensitive conduction speed	31.493	35.167	43.133*	47.673

*p < 0.05 *vs* surgery treatment

Similar results were obtained in the examinations of the sensitive conduction speed recovery of the median nerve. During the initial measurements, before the beginning of the treatment, almost identical values of the sensitive conduction speed (SCS) were registered in both groups of the patients (SCS1 = 32.200 msec and SCS2 = 31.493 msec). The measurements performed at the 30th postoperative day, revealed statistically much better recovery in the group of the patients treated with betamethasone applied intraoperatively (t = -2.516; p = 0.018). In patients of the second group almost full recovery of the sensitive conduction speed was achieved already after 30 days (mean value SCS2 = 43.133 msec). Mean values at the final measurements did not show a statistically important difference between treatment methods applied (SCS1 = 45.347 msec in the first group and SCS2 = 47.673 msec in the second group of the patients).

Discussion

The aims of CTS treatment today are not the same as used to be decades ago. In fact the only aim of treatment before was to achieve vanishing of compressive neuropathy symptoms, a subjective good feeling and satisfaction of the patients. Today, the aim of the treatment is not only to resolve the present symptoms, but to find a way of the best and the fastest restitution of all hand functions, and those of the compressed nerves.

The initial therapy for the majority of CTS patients is nonsurgical one [14, 18]. In 1966 Phalen [13] thought that the majority of CTS patients should not undergo surgical treatment. Surgeons who treat hand diseases, often start a treatment with practicing one of nonsurgical methods during a few weeks, and make a decision afterwards whether to go for a surgical treatment [17].

Investigations done by Katz and Simmons [14], showed that the improvement regarding the before-surgery symptoms like pain and numbness tend to go much more quickly than the recovery of the motion and sensitive symptoms. The same authors reported the fact that locally applied corticosteroid injections provided faster recovery of the median nerve conduction.

According to the up-to-date knowledge, a preoperative application of corticosteroids results in a better surgery outcomes of the CTS. In the patients who, in the course of their nonsurgical treatment, have been treated with corticosteroid injection, a temporary improvement and relieving of symptoms, as well as far better surgery outcomes are achieved. Marshall et al. [16] reported better surgery outcome in 94% of patients treated with corticosteroids before surgery with registered temporary relieving of symptoms.

There is no doubt that a surgical treatment is far more efficient way to resolve the CTS, than a conservative one. Wishing to make a surgical treatment more efficient by using positive outcome of the steroid injection in the treatment of the CTS, we examined the possible effects of the steroids on the time needed for the recovery of the median nerve conduction, in the surgically treated CTS patients.

We were surprised with the results at the measurements of the median nerve conduction speed in the carpal tunnel. Significantly better results were reached in the patients treated with betamethasone.

Examination of a recovery of the sensitive conduction speed showed as well, that the degree of recovery was far higher in the second group of patients, and the obtained difference in the values was statistically significant after thirty days, while the values obtained at the final measurements, ninety days after, were almost identical. Consequently, the injected betamethasone did not produce better, but faster recovery.

The obtained results demonstrate, without doubt, the positive effects of injecting steroids within the surgical decompression. It ought to be pointed out that a full recovery of the compressed nerve conduction was achieved in both groups of the patients. However, in both examined variables at different time intervals, we got, to a certain point faster and better recovery of the examined ED parameters in the second group of the patients were given 1 ml of betamethasone within the surgical procedure.

Conclusion

The results obtained showed that intraoperative application of the steroid injection during the surgical decompression resulted in faster regaining of the median nerve conduction speed.

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Myocardial hipertrophy in hypertensive patients with and without metabolic syndrome

Hipertrofija miokarda kod bolesnika sa arterijskom hipertenzijom sa metaboličkim sindromom i bez njega

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Abstract

Background/Aim. Beside arterial hypertension as the most important factor of a myocardial hypertension development, very important risk factors are obesity, hypercholesterolemia, insulin resistance, etc. The aim of the study was to examine the influence of metabolic syndrome (MetS) on left ventricular hypertrophy in patients with arterial hypertension. **Methods.** We checked medical records for 138 patients with arterial hypertension, and compared them with the control group of 44 normotensive subjects. The patients with arterial hypertension were divided into two groups considering the presence of MetS: with MetS (59 patients), and without MetS (79 patients). We defined MetS as presence of three (or more) within five criteria: central obesity (> 102 cm male, > 88 cm female), raised triglycerides (> 1.7 mmol/L, or drug treatment for elevated triglycerides), reduced high density lipoprotein (HDL) cholesterol (< 1.03 mmol/L male, < 1.3 mmol/L female), raised blood pressure (> 130 mmHg systolic, > 90 mmHg diastolic), raised fasting glucose (> 6.11 mmol/L, or drug treatment for elevated glucose level). In each group routine laboratory, echocardiography and 24-hour ambulatory blood pressure monitoring were performed. **Results.** We found statisticaly significant higher left ventricular mass in both subgroups hypertensive patients in comparison with the control group ($p < 0.05$). We did not find statistically significant difference (227.31±63.44 *vs* 219±59.5, $p > 0.05$) in left ventricular mass between these two groups of patients. In the patients with arterial hypertension and MetS we found hypertrophy more frequently than in the subgroup without MetS (43/57 *vs* 34/69, $p < 0.001$). **Conclusion.** Our results suggest that associated cardiometabolic risks increase the prevalence of myocardial hypertrophy, but do not influence left ventricular mass.

Key words:
metabolic syndrome X; hypertrophy, left ventricular; hypertension; risk factors; risk assessment.

Apstrakt

Uvod/Cilj. Pored arterijske hipertenzije, koja je najvažniji faktor rizika u nastanku hipertrofije miokarda, veoma važni su i gojaznost, hiperholesterolemija i insulinska rezistencija. Cilj rada bio je da se ispita uticaj metaboličkog sindroma (MetS) na razvoj hipertrofije miokarda kod bolesnika sa arterijskom hipertenzijom. **Metode.** U ispitivanje je bilo uključeno 138 bolesnika sa arterijskom hipertenzijom i 44 normotenzivna ispitanika koji su činili kontrolnu grupu. Bolesnici sa arterijskom hipertenzijom podeljeni su, na osnovu pridruženih kardiometaboličkih faktora rizika karakterističnih za MetS, u podgrupu bolesnika sa arterijskom hipertenzijom u sklopu MetS (n = 59) i drugu podgrupu sa izolovanom arterijskom hipertenzijom (n = 79 bolesnika). Na osnovu postojanja tri ili više kriterijuma definisali smo MetS, s tim što je obavezni kriterijum bila arterijska hipertenzija (sistolni pritisak > 130 mmHg, dijastolni > 90 mmHg). Preostali kriterijumi korišćeni za dijagnozu MetS bili su: nivo triglicerida u serumu > 1,7 mmol/l, nivo lipoproteina velike gustine (HDL) u serumu < 1,30 mmol/l za žene i < 1,04 mmol/l za muškarce, nivo glikemije > 6,11 mmol/l, obim struka > 88 cm za žene i > 102 cm za muškarce. Kod svih bolesnika određene su vrednosti arterijskog pritiska 24-časovnim ambulantnim monitoringom, urađene su standardne laboratorijske analize i načinjen je ehokardiografski pregled. **Rezultati.** Nađena je statistički značajno veća masa leve komore u obe podgrupe bolesnika sa arterijskom hipertenzijom u poređenju sa kontrolnom grupom ($p < 0,05$). Nije nađena statistički značajna razlika u masi leve komore (227,31±63,44 *vs* 219±59,5 $p > 0,05$) između podgrupa bolesnika sa arterijskom hipertenzijom sa i bez MetS. Kod bolesnika sa arterijskom hipertenzijom i pridruženim kriterijumima za MetS nađena je statistički značajno veća učestalost hipertrofije nego u podgrupi bolesnika sa arterijskom hipertenzijom bez pridruženih metaboličkih poremećaja (43/59 *vs* 34/79, $p < 0,001$). **Zaključak.** Pridruženi kardiometabolički faktori rizika povećavaju učestalost hipertrofije, ali ne utiču značajnije na porast mase leve komore.

Ključne reči:
metabolički sindrom X; hipertrofija leve komore; hipertenzija; faktori rizika; rizik, procena.

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Introduction

Myocardial hypertrophy is a chronic left ventricular (LV) adaptation caused by increased burden of pressure or volume. These two hemodynamic factors are crucial for molecular changes that take part in cascade reactions, which are necessary for compensatory effects. Increasing wall stress and stretching are the stimuli for transcriptional messenger ribonucleic acid (mRNA) and increasing protein level in the cardiomyocites.

Numerous factors participate in myocardial LV hypertrophy appearance and development. Arterial hypertension has the main role, but obesity, insulin resistance, hypercholesterolemia, a salt-rich diet, terminal renal failure, anemia, etc. also play an important role [1].

Some of these metabolic disorders are most important in the pathogenesis of metabolic syndrome (MetS). This disease changed its name few times in the past – insulin resistance syndrome, X-syndrome, deadly quartet and dysmetabolic syndrome are all the old terms used for MetS. About ten years ago, World Health Organization (WHO) defined MetS and put emphasis on its clinical importance and possible consequences. Today we use two formal definitions of this disease. According to The National Cholesterol Educational Program Third Adult Treatment Panel (NCEP-ATP III) MetS is defined as the presence of three or more of five abnormalities: glucose metabolism disorder, arterial hypertension, hypertriglyceridemia, low high density lipoprotein (HDL) cholesterol level and central obesity [2]. Other definition, created by the International Diabetes Federation (IDF), is based on the presence of central obesity, together with two (out of remaining four) criteria [3].

Negative influence of the presence of MetS in cardiovascular patients was proven. Screening of subclinical organ damage could be very useful for cardiovascular risk evaluation – including LV hypertrophy.

The aim of the study was to examine the influence of the MetS on LV hypertrophy development in patients with arterial hypertension, without any clinical manifestation of cardiovascular disease.

Methods

This study included 182 patients, divided into two groups. The first group consisted of 138 patients (72 females and 66 males) with arterial hypertension. The other group contained 44 patients (24 females and 20 males) with normal blood pressure values, a maximum of one (other) criteria for MetS and no evidence of any cardiovascular diseases. They were used as controls.

Our criteria for including patients in the group I were: the presence of hypertension for no more than three years with ambulatory measured values (3 times in 7 days) higher than 130/90 mmHg, or normotensive under antihypertensive therapy. We included patients without any clinical or laboratory signs of heart failure, coronary disease, stroke, valvular diseases, any cause of (possible) secondary hypertension or other chronic diseases such as cirrhosis, renal failure or endocrine diseases.

Monitoring was performed with a mercury sphygmomanometer: each patient and member of the control group had their blood pressure values measured twice (with a 5-minute pause) in the morning (the average value of these two results was used). For 24-hour ambulatory monitoring values, we used the average 24-hour values of systolic and diastolic blood pressure values.

The following laboratory tests were also performed: glucose level, total cholesterol level, HDL cholesterol level and triglycerides level.

Body weight and height were measured in the morning. The body mass index was calculated as the individual's body weight divided by the square of their height. The waist circumference at the end of normal expiration was measured while the person was standing at the midway between the lower costal margin and the iliac crest.

The criteria for the selection of patients with MetS was the presence of hypertension together with two or more criteria of the following:

– triglycerides level equal to or higher than 1.7 mmol/l;

– HDL cholesterollevel lower than 1.30 mmol/l for women and 1.04 mmol/l for men;

– glucose level equal to or higher than 6.11 mmol/l;

– waist circumference over 88 cm (women) and 102 cm (men).

The patients with arterial hypertension were divided into two subgroups: one consisting of the patients with hypertension but without MetS - 79 patients, and the other one consisting of the patients who had hypertension as a part of MetS - 59 patients.

The end-diastolic and end-systolic diameters, LV free wall thickness and interventricular septal thickness were calculated according to the recommendations of the American Society of Echocardiography [4]. The end-diastolic and end-systolic volumes, and the systolic function parameters – ejection fraction (EF) and fractional shortening (FS) were calculated using the Teicholz formula.

The LV mass was calculated using the Penn formula: LV mass (Penn) = 1.04 (LVIDD + PWTD + $[IVSTD]^3$ – $[LVIDD]^3$) – 13.6 (LVIDD – endiastolic diameter of the LV, the PWTD – LV free wall thickness and the IVSTD – interventricle septal thickness) [5]. The LV mass index (indexation of LV mass) was calculated according to the body surface area (BSA).

The criteria for myocardial hypertrophy were LVmass/BSA values ≥ 116 g/m^2 (male) and 104 g/m^2 (female).

Relative ventricular wall thickness (RWT) = 2 PWTD/LVIDD.

Descriptive and analytic (Chi-square and *t* tests) was expressed as RWT statistics were used for data analysis.

Results

Age and gender were compatible in all the groups examined.

Glucose, triglycerides and HDL cholesterol levels, body weight and body mass index are presented in Table 1.

Glucose and triglycerides levels were statistically significant in the group of patients with hypertension as a part of MetS compared with patients who had isolated hypertension ($p < 0.05$). The level of HDL cholesterol was slightly lower in the group of hypertensive patients with MetS compared with the patients with isolated hypertension and the control group, but without statistical significance ($p > 0.05$). The body mass index and waist circumference were statistically significantly higher in the group of patients with MetS compared with both the patients with both hypertension and the control group ($p < 0.05$).

The average end-diastolic and end-systolic diameters of the LV were in the range of physiological values in both groups, without any significant discord compared to the control group, IVSTD was significantly higher in all patients with hypertension, regardless of whether it was part of the MetS or not. PWDT was higher in both subgroups compared to control group, but without significant variation between the subgroups with/without MetS.

The values of the EF and FS parameters were in the range of the physiological values in all the groups, without any significant discord.

Table 1

Characteristics of hypertensive patients with and without metabolic syndrome (MetS) and normotensive control

Parameter	Control group ($\bar{x} \pm SD$)	Patients with MetS ($\bar{x} \pm SD$)	Patients without MetS ($\bar{x} \pm SD$)
Age (year)	47±4	51 ± 4	53 ± 5
Body mass index	23.8±3.8	30.8 ± 3.7	26.1 ± 3.4†
Waist circumference, male (cm)	82±7	109±8*	97±9*, †
Waist circumference, female (cm)	79±8	98±8*	88±7*, †
Glucose level (mmol/l)	4.84±0.7	5.37±0.8*	4.92±0.7†
HDL, male (mmol/l)	1.68±0.4	1.09±0.2	1.59±0.3
HDL, female (mmol/l)	1.71±0.3	1.32±0.3	1.45±0.4
Triglycerides (mmol/l)	0.98±0.7	2.2±0.7*	1.87±0.7*, †
Heart rate (n/min)	71±6	86±9.2	80±8.6
SAP (mmHg)	118±7.81	152±10.42*	150±9.89*
DAP (mmHg)	76±5.7	102±6.4	99±5.2
24h SAP (mmHg)	115±7.80	146±10.52*	142±11.20*
24h DAP (mmHg)	75±5.2	98±6.4*	96±5.4*
24h heart rate (n/min)	72±7	86±8.7	80±8.6

*$p < 0.05$ *vs* the control group; †$p < 0.05$ *vs* patients with MetS
HDL – high density hipoprotein cholesterol
SAP – systolic arterial pressure
DAP – diastolic arterial pressure

Systolic and diastolic isolated pressure values and heart rate measured in ambulance, and also the values from 24-hour ambulatory monitoring, are presented in Table 1. It is obvious that blood pressure values were higher in both groups of patients, compared with the control group. There was no significant difference in these values between the two groups of patients ($p > 0.05$).

Table 2 shows echocardiography parameters in the groups of patients observed.

The values of the RWT were statistically significantly higher in the patients with hypertension (regardless of the subgroup) compared to the control group .

The average LV mass values were significantly higher in patients with raised blood pressure compared to the control group – regardless of the presence of MetS ($p > 0.05$).

The LVmass/BSA showed a significant frequency of hypertrophy in the patients with MetS (43/57 *vs* 34/69, respectively; compared with patients with isolated hypertension ($p < 0.001$).

Table 2

Echocardiography (ECHO) parameters in hypertensive patients with and without metabolic syndrome (MetS) and normotensive control

ECHO parameters	Control group ($\bar{x} \pm SD$)	Patients with MetS ($\bar{x} \pm SD$)	Patients without MetS ($\bar{x} \pm SD$)
LVIDD (cm)	4.54 ± 0.4	4.79 ± 0.3	4.78 ± 0.2
LVISD (cm)	2.9 ± 0.2	3.2 ± 0.2	2.9 ± 0.2
Interventricular septum (cm)	0.8 ± 0.04	1.13 ± 0.06	1.08 ± 0.04*
Posterior wall (cm)	0.8 ± 0.02	1.07 ± 0.04*	1.07 ± 0.04*
Ejection fraction (%)	65.04	63.72	64.45
Fractional shortening (%)	33.74	34.02	33.59
Left ventricular mass (g)	129.81 ± 22.24	227.31 ± 63.44*	219.02 ± 59.5*
RWT	0.35 ± 0.02	0.44 ± 0.03*	0.44 ± 0.03*

* $p < 0.05$ *vs* the control group; LVIDD – left ventricular internal dimension in diastole; LVISD – left ventricular internal systolic diameter; RWT – relative ventricular wall thickness

Discussion

In the patients with hypertension, with or without MetS, PWDT and IVSTD, RWT and LV mass were all significantly higher as compared to normotensive people. At the same time, there was no significant difference of these parameters between two subgroups of hypertensive patients (with or without MetS). That unmistakably demonstrated the central role of hypertension in the (patho)genesis of these structural changes. Human hypertension has an important attribute: pressure burden or volume burden causes various adaptations of the heart LV. According to epidemiologic information – the prevalence of concentric heart remodelling and concentric myocardial hypertrophy – the conclusion is that a pressure burden is the main abnormality in arterial hypertension, with volume burden as an associated component. The cellular adaptation basis in a pressure burden is based on the sarcomeres and extracellular changes, which are more pronounced in this process than in the case of volume burden. In case of an additional volume burden present (with a dominant pressure burden) it is common to see collagen accumulation – though this process does not appear to be important in causing concentric remodelling and concentric myocardial hypertrophy [6].

An important difference between our subgroups of hypertensive patients is the significant occurrence of myocardial hypertrophy in the patients with MetS. The absence of (significant) differences between blood pressure values in the subgroups of patients rules out the influence of the blood pressure level on the frequency of myocardial hypertrophy. A number of studies have suggested that hypertrophy is more frequent in hypertensive patients with MetS as compared to the patients with hypertension without MetS [7–12].

In the PAMELA study [13], normotensive patients with MetS were examined and an increased prevalence of elevated values for LVmass index and myocardial hypertrophy was found [3]. One of the conclusions was that changes in the heart structure connected were not only with hypertension – metabolic and neural components of this syndrome were important, too. The role of sympathetic stimulation (their influence on cardiomuscular hypertrophy and connective tissue proliferation) was emphasised. It is likely that other metabolic factors will be considered as responsible for a higher frequency of myocardial hypertrophy.

Different levels of carbohydrate metabolism disturbance – glucose metabolism disorder, diabetes mellitus type II and insulin resistance are the cause of LVmass increase [14]. *In vitro* (animal and human model) effects of insulin are salt retention and sympathetic stimulation, as well as peripheral vascular resistance. The first two mechanisms could be seen as responsible for increasing the LVmass index. Concentric hypertrophy in patients with insulin resistance could be explained by vasoconstriction and pressure burden.

An important negative effect of obesity on the cardiovascular system is hypertrophy of the left side of the heart. Volume burden is a characteristic of this, so it was thought that the manifestation should be eccentric hypertrophy of the LV. However, echocardiography studies showed different structural changes. Avelar et al. [15] suggested that both patterns of hypertrophy could be found in overweight people – but concentric hypertrophy was more frequent. Indirectly, they came to the conclusion that sympathetic stimulation together with hypertension are responsible for this finding. They also found that obstructive sleep apnoea leads to hypertrophy development in obesity - because there is intermittent hypoxia during the obstruction of the airways and increased sympathetic tone with a consequential increase in heart frequency and blood pressure.

It is a premise that increasing levels of triglycerides in blood could be a reason for structural and functional changes in the LV, by increasing the influx of fatty acids in the myocites. Investigation of this hypothesis showed that there were no significant changes in structure, but signs of diastolic dysfunction were found in this metabolic disorder [16].

Here we mentioned data on the influence of the single risk factors (in MetS) on the development of myocardial hypertrophy as a subclinical form of heart damage. The global influence of the MetS has been shown through linear association between the number of risk factors and the prevalence of target organ damage and cardiovascular complications [17]. Grandi et al. [12] showed the dominant effect of the 24-hour average values of systolic pressure and body mass index on the development of LV hypertrophy in patients with MetS without diabetes. Palmieri and Bella [18] considered that the results of Grandi's investigation emphasised insulin resistance as an important additional factor in hypertrophy in patients without diabetes.

Conclusion

The results of this investigation suggest that arterial hypertension has a leading role in the development of myocardial hypertrophy, both in patients with and without MetS. Other components of MetS are important in increasing the occurrence of LV hypertrophy. Myocardial hypertrophy is an intermediary marker of heart damage and an important predictor of heart failure, coronary disease and stroke. Because of that, therapy for hypertension and also other components (obesity and insulin resistance above all) is obligatory.

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C U R R E N T T O P I C S

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Histological, mucinohistochemical and immunohistochemical features of gastric signet ring cell carcinoma

Histološke, mucinohistohemijske i imunohistohemijske karakteristike *signet ring cell* karcinoma želuca

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Key words:
carcinoma, signet ring cell; mucins; histology; immunohistochemistry; phenotype.

Ključne reči:
želudac, neoplazme; mucini; histologija; imunohistohemija; fenotip.

Introduction

Gastric cancer is the fourth most common cancer and the second cause of cancer-related death worldwide [1–3]. Various types of gastric cancers differ in their epidemiology, pathogenesis, genetic profile and clinical outcome [4]. There has been the overall decline in the total incidence of gastric cancer in the last few decades worldwide, but the decline of signet ring cell (SRC) type of gastric carcinoma has been more gradual and some studies have even reported an increase of SRC type carcinoma [5]. As a result, there are different data about incidence of SRC carcinoma - it has been reported that 3.4 – 29% of patients with gastric cancer had SRC type histology and the newest data say that SRC carcinoma accounts for more than 30% of gastric carcinomas in some reported series [4–7].

Comparing with other types of gastric cancer, SRC gastric carcinoma has a tendency to involve the entire stomach and to extend directly into neighboring organs. It has the poorest prognosis, is more common in females than males and occurs at a comparatively younger age [8]. Although most researchers believe that SRC carcinoma is characterized by poor differentiation, strong invasive tendency and poor prognosis, the clinicopathologic parameters of this type of malignancy are still controversial [8–10]. Controversial reported data on gastric SRC cancer incidence, prognosis, histogenesis, metastasis, phenotipic histologic and immunohistochemical features, and confusion induced by the presence of morphologic patterns other than the conventional appearance, are the facts that require further investigations [8–15].

Classifications of gastric carcinoma

Histologic classifications

Gastric carcinomas have been classified into two main histologic subtypes by standard hematoxylin-eosin staining according to their gland-forming tendencies, intestinal and diffuse type according to Lauren [16], which essentially correspond to the differentiated and undifferentiated types, respectively, according Nakamura et al. [17].

Intestinal carcinoma was considered to be almost equal to differentiated carcinoma, and diffuse carcinoma was considered almost equal to gastric or undifferentiated carcinoma [18].

Neoplasms that contain approximately equal quantities of intestinal and diffuse components are called mixed carcinomas. Carcinomas too undifferentiated to fit neatly into either category are placed in the indeterminate category [16].

Intestinal carcinomas form recognizable glands that range from well differentiated tumors, sometimes with poorly differentiated tumor at the advancing margin. They typically arise on the background of intestinal metaplasia [19].

Diffuse carcinomas consist of poorly cohesive cells diffusely infiltrating the gastric wall with little or no gland formation. The cells usually appear round and small, either arranged as single cells or clustered in abortive, lacy gland-like or reticular formations. These tumors resemble those classified as SRC tumors in the Wored Health Organisation (WHO) classification. The mitotic rate is lower in diffuse carcinomas than in intestinal tumors. Desmoplasia is more pronounced and associated inflammation is less evident in diffuse cancers than in the intestinal carcinomas [19].

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With respect to histogenesis of these two types of gastric carcinoma, intestinal/differentiated type tumors have generally been considered to arise from the gastric mucosa with intestinal metaplasia and diffuse/undifferentiated type tumors from the ordinary gastric mucosa without intestinal metaplasia, and the two are considered to follow different genetic pathways during carcinogenesis [16–21].

However, recent reports have shown that gastric and intestinal phenotypic cell markers are widely expressed in gastric carcinomas, irrespective of their histological type [22–25].

World Health Organization classification

This classification is based on the predominant histological pattern. More than 50% of SRC carcinomas consist of isolated or small groups of malignant cells containing intracytoplasmic mucin. Superficially, SRC lie scattered in the lamina propria, widening the distances between the pits and glands. The tumor cells have five morphologies: nuclei push against cell membranes creating a classical signet ring cell appearance due to an expanded, globoid, optically clear cytoplasm [26]. These contain acid mucin and stain with Alcian blue at pH 2.5 (Figure 1); other diffuse carcinomas contain

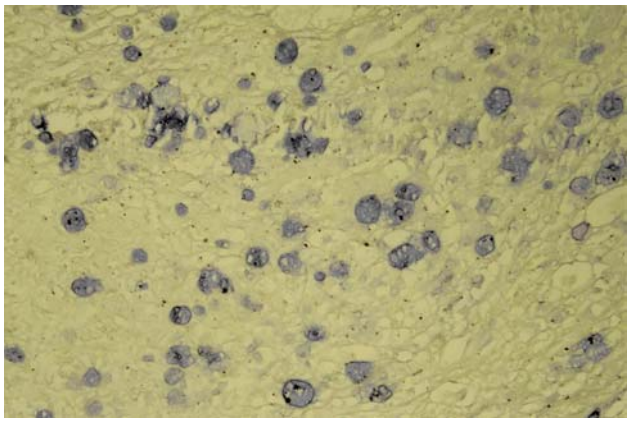

Fig. 1 – Signet ring cell gastric carcinoma – Hypersecretion of intestinal (acid) mucin (HID-AB, pH=2, 5; × 400)

cells with central nuclei resembling histiocytes, and show little or no mitotic activity; small deeply eosinophilic cells with prominent, but minute cytoplasmic granules containing neutral mucin; small cells with little or no mucin, and anaplastic cells with little or no mucins (Figure 2). These cell types intermingle with one another and constitute varying tumor proportions. SRC tumors may also form lacy or delicate trabecular glandullar patterns and they may display a zonal or solid arrangement.

Signet ring cell carcinomas are infiltrative; the number of malignant cells is comparativelly small and desmoplasia may be prominent. Histochemical stains, including mucin stains (PAS, Alcian blue, HID-AB at pH 2.5) or immunohistochemical staining with antibodies to cytokeratin, help detect sparsely dispersed tumor cells in the stroma. Cytokeratin immunostains detect a greater percentage of neoplastic cells than do mucin stains (Figure 3).

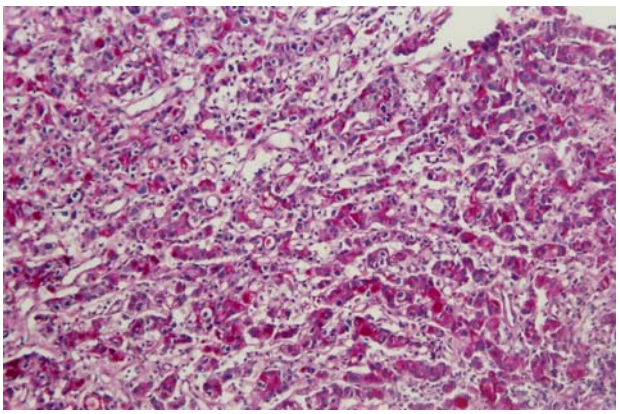

Fig. 2 – Signet ring cell gastric carcinoma – Hypersecretion of gastric neutral mucin (AB-PAS, pH=2, 5; × 200)

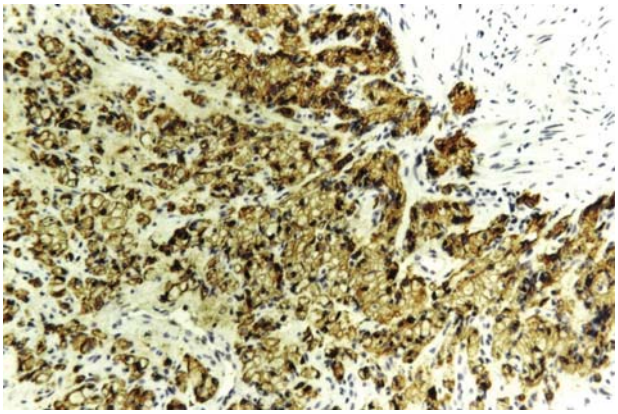

Fig. 3 – Signet ring cell gastric carcinoma – Strong diffuse expression of pancytokeratin (LSAB2; × 200)

Several conditions mimic SRC carcinoma including signet ring lymphoma, gastric mucosa associated lymphoid tissue (MALT) lymphoma, gastrointestinal stromal tumor (GIST), lamina propria muciphages, xantomas and detached or dying cells associated with gastritis [15, 26].

Phenotypic classification

Mucin histochemical and immunohistochemical methods enabled phenotypic classification of gastric cancers based on mucin expression profile.

Mucins are high molecular weight heavily O-glucosylated glycoproteins produced by secretory epithelial cells, that have many physiologic roles in normal tissues [12, 27, 28]. Specific types of mucin are individually referred to as MUC and designated with a number representing the order in which the mucin was described. Secretory mucins (MUC2, MUC5AC, MUC5B, and MUC6) act as a first-line defense as physical protective barriers for epithelial surfaces. Another subset of mucins, transmembrane type (MUC1, MUC3A, MUC3B, MUC4, MUC12, and MUC17), may serve as ligands and modulators in cell signaling [28]. Mucins have many physiologic functions as well as distinct pathologic changes in tumor and metastasis. Several protective functions as well as additional pathologic mechanisms of mucins have been proposed in cancer including protection

from host response, decreased cell adhesion, tumor invasion, and other changes in metastatic potential [28]. These two families of mucins, secretory and transmembrane, represent most of the clinically relevant mucins that have been described [27].

In gastric mucosa, two types of mucus-secreting cells exist: the surface mucous cells and gland mucous cells (which includes cardiac gland cells, mucous neck cells, and pyloric gland cells) [12].

The mucin expression pattern of gastric carcinoma is heterogeneous. It includes mucins normally expressed in gastric mucosa – gastric phenotypic markers (MUC1, MUC5AC and MUC6) and *de novo* expression of the intestinal mucin – intestinal phenotypic marker MUC2 [11, 27].

According to the expression of phenotypic markers, SRC carcinomas are classified into four differentiated phenotypes [22]. G type (tumors that are positively stained by one or more gastric phenotypic markers, but no intestinal phenotypic marker); I type (those stained by one or more intestinal phenotypic markers, but no gastric marker); GI (mixed) type (those positively stained by both gastric and intestinal phenotypic markers) and UC (unclassified type) (those stained by none of the phenotypic markers) (Figures 4–6).

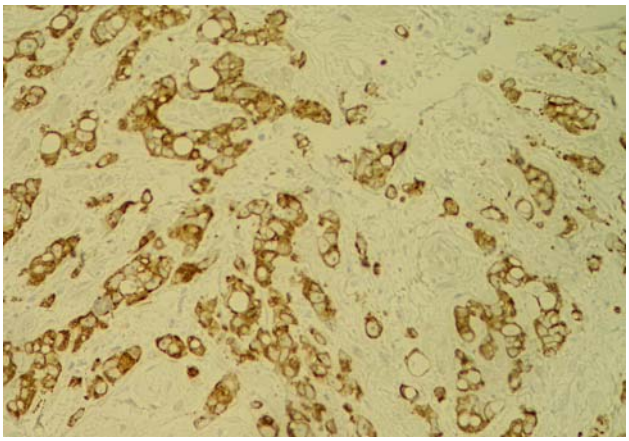

Fig. 4 – Signet ring cell gastric carcinoma – Intensive expression of MUC6 (LSAB2; × 400)

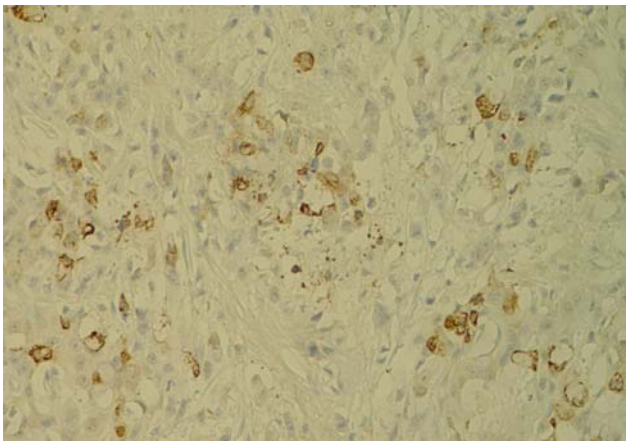

Fig. 5 – Signet ring cell gastric carcinoma – Multifocal weak expression of MUC2 (LSAB2; × 400)

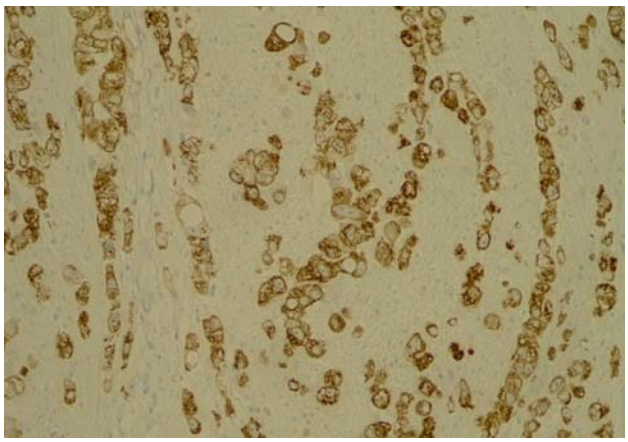

Fig. 6 – Signet ring cell gastric carcinoma – Diffuse intensive expression of MUC5AC (LSAB2; × 400)

Signet ring cell carcinoma can be easily missed on initial microscopic examination due to inconspicuousness of the tumor cells and marked desmoplasia. In addition, the WHO classification provides five morphologic patterns of tumor cells, other than the conventional appearance, inducing a great diagnostic difficulty. This different morphology, and possible similarity with epitheloid GIST, hepatoid variant of gastric carcinomas, MALT lymphomas, xantomas, and marked inflammatory and desmoplastic reaction (coupled with the inconspicuousness of the tumor cells) enter the list of diagnostic possibilities. However, the histochemistry for mucin and immunohistochemical positivity for panCytokeratin and negativity for CD117, SMA, S-100 protein, CD20 and CD45Rö, exclude this diagnostic possibility. In addition to, specific antibodies to the various kinds of mucins (MUC1, MUC2, MUC6, MUC5AC, MUC10) are also used to define gastric and intestinal phenotypes, to provide new insights in the differentiation pathways of the gastric carcinomas.

A number of clinical studies revealed the difference in biological behaviors and prognosis among patients with gastric SRC carcinoma, indicating that morphologic classification is not enough to predict the progression and outcome of this kind of gastric carcinoma, and subtype classification needs further investigations [9, 22, 25]. Our previous study showed that different phenotipic expression patterns were significantly associated with clinicopathologic parameters and prognosis of SRC carcinoma of the stomach [29–32].

Conclusion

Numerous morphologic variations of SRC carcinoma pose an important diagnostic dilemma.

Various epithelial, stromal and lymphomatous tumors, and xantomatous gastritis enter the list of diagnostic possibilities. The accurate diagnosis is essential for therapeutic and prognostic considerations.

Immunohistochemistry is “gold standard” for SRC carcinoma diagnosis.

Examination of phenotype expression may be useful evidence for further classification and prognostic prediction in gastric SRC carcinomas.

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K A Z U I S T I K A

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Serozni mikrocistični adenom glave pankreasa kao uzrok bilijarne opstrukcije

Serous microcystic adenoma of the head of the pancreas causing an obstructive jaundice

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Apstrakt

Uvod. Serozni mikrocistični adenom pankreasa redak je benigni tumor egzokrinog pankreasa koji vodi poreklo od epitela izvodnih kanala. Izgrađen je od velikog broja malih cisti obloženih kuboidnim ćelijama, ispunjenih bistrom seroznom tečnošću i razdvojenih fibrokolagenom stromom. Javlja se najčešće kod žena u 7. i 8. deceniji, na distalnom pankreasu i ima veoma malu sklonost ka malignoj alteraciji. Kod 2/3 bolesnika simptomi su nekarakteristični, a kod 1/3 odsutni. Kad je lokalizovan u glavi pankreasa, tumor veoma retko dovodi do opstruktivnog ikterusa. **Prikaz bolesnika.** Prikazana je bolesnica, stara 61 godinu, koja je imala višemesečne blage i nespecifične tegobe u trbuhu, a na kraju se ispoljila i bezbolna progresivna, opstruktivna žutica. Tokom operacije nađen je policistični tumor glave pankreasa koji je kompresijom i dislokacijom portomezenterične vene doveo do venske staze. Tumor je odstranjen pilorus prezervirajućom cefaličnom duodenopankreatektomijom po Whippleu (modifikacija po Longmire-Traversou). Histološkim pregledom dokazan je serozni mikrocistični adenom pankreasa. Posle normalnog postoperativnog toka bolesnica nije imala tegobe. **Zaključak.** Iako je veoma redak, serozni mikrocistični adenom mora biti razmatran u diferencijalnoj dijagnozi cističnih lezija glave pankreasa. Veoma retko može dovesti do opstruktivne žutice. Izlečenje se postiže resekcijom, koja može biti i zahtevna.

Ključne reči:
pankreas, egzokrini; adenom; dijagnoza; dijagnoza, diferencijalna; žutica, opstruktivna; pankreatikoduodenektomija.

Abstract

Background. Serous microcystic adenoma is a rare benign tumor of the exocrine pancreas originating from the ductal system and composed of a large number of small cysts covered by cuboid cells, filled with clear serous fluid and separated with fibrocolagenous stroma. Most frequently it appears in women in 7th and 8th decades, in the distal pancreas. It shows a very low malignant potential. In 2/3 of patients symptoms are uncharacteristic and in 1/3 they are absent. When localised within the head of the pancreas it rarely causes an obstructive jaundice. **Case report.** We presented a 61-year-old female patient who for months had had mild and nonspecific abdominal symptoms developing to progressive obstructive jaundice. At surgery we revealed a rather large policystic mass of the head of the pancreas causing not only obstructive jaundice but also a venous stasis by compression and dislocation of the portomesenteric vein. The tumor was removed with pylorus preserving cephalic duodenopancreatectomy (Whipple's procedure modified by Longmire-Traverso). Histology confirmed serous microcystic adenoma of the pancreas. The postoperative recovery was uneventful and preoperative symptoms disappeared. **Conclusion.** Although very rare, serous microcystic adenoma might appear within the head of the pancreas and has to be taken into consideration in differential diagnosis of cystic lesions of the head of the pancreas. Very rarely the tumour might cause obstructive jaundice. Surgical resection, which might be demanding, leads to complete recovery .

Key words:
pancreas, exocrine; adenoma; diagnosis; diagnosis, differential; jaundice, obstructive; pancreaticoduodenectomy.

Uvod

Serozni mikrocistični adenom pankreasa (SMAP) (sinonimi: mikrocistični serozni cistadenom, serozni cistadenom, glikogenom bogati cistadenom) je benigni tumor pankreasa. Sastoji se od velikog broja malih cisti koje sadrže bistru tečnost, obloženih svetlim kuboidnim ćelijama i razdvojenih fibrokolagenom stromom [1, 2]. Tumor je redak i čini 1–2% svih egzokrinih tumora pankreasa [3]. Smatra se da vodi poreklo od epitela izvodnih kanala egzokrinog pankreasa [4]. Raste sporo, pa su simptomi najčešće u vezi sa tzv. *mass* efektom tumora [5]. Kod dve trećine bolesnika vodeći simpto-

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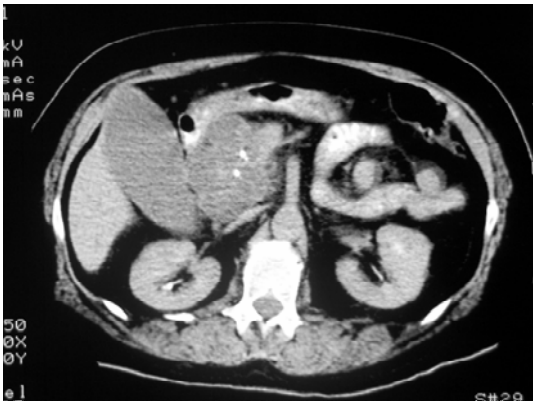
mi su bol u trbuhu, palpabilan tumor, muka, povraćanje i smanjenje telesne mase. Kod trećine bolesnika tumor se otkriva slučajno, tokom pregleda preduzetog zbog drugog razloga ili na autopsiji [6].

I kad je lokalizovan u glavi pankreasa, retko dovodi do bilijarne opstrukcije [5]. Kod prikazane bolesnice SMAP glave pankreasa doveo je do opstruktivne žutice.

Prikaz bolesnika

Bolesnica, stara 61 godinu, sa arterijskom hipertenzijom, tokom poslednjih meseci imala je povremene bolove u leđima. Dva meseca pre prijema u našu ustanovu urađeni su joj ultrazvučni pregled (US) i kompjuterizovana tomografija (KT) na kojima su viđeni solidnocistična tumefakcija glave pankreasa promera 7 × 6 cm (slika 1) i dilatacija žučne kese i celog intra- i ekstrahepatičnog bilijarnog stabla. Nekoliko dana pre prijema u našu ustanovu bolesnica je požutela. Klinički, palpirana joj je umereno napeta žučna kesa, a medijalno od nje, u epigastrijumu neravna, skoro potpuno nepokretna tumefakcija. Od normalnih vrednosti odstupali su: bilirubin (148 µmol/l), direktni bilirubin (78,6 µmol/l), alkalna fosfataza (393 IJ/l), gama GT (2 321 IJ/l), SGOT (345 IJ/l), SGPT (671 IJ/l), sedimentacija (41 mm/h).



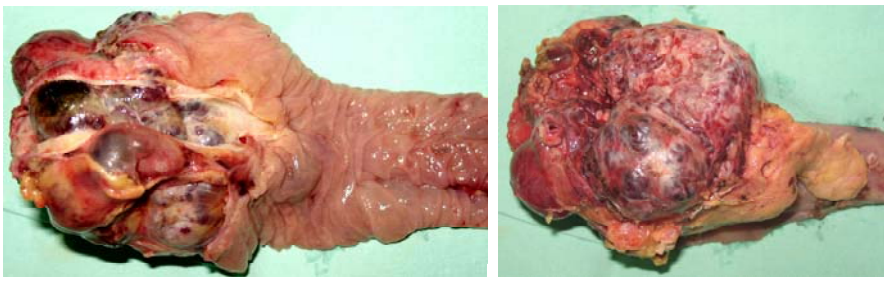
Sl. 1 – Kompjuterska tomografija otkriva solidnocističnu tumefakciju glave pankreasa

tiranim Wirsungovim kanalom. Tumor je obuhvatao portnu venu sa desne, prednje i zadnje strane, ali je nije infiltrisao. Uz dosta teškoća tumorska masa je odvojena od portne i mezenterične vene i učinjena je cefalična duodenopankreatektomija po Whippleu sa prezervacijom pilorusa po Longmire-Traversu. Zbog nemogućnosti da se isključi malignitet načinjena je standardna limfadenektomija.

Resekcioni preparat sastojao se od duodenuma bez bulbusa i glave pankreasa koja je bila skoro kompletno tumorski promenjena. Duktus holedohus bio je dug 82 mm, sa ekstrapankreatičnim delom dužine 30 mm i pri proksimalnoj liniji resekcije obima 47 mm. Papila Vateri, promera 7 mm i udaljena 45 mm od proksimalne linije resekcije, bila je makroskopski neizmenjena.

Na spoljnoj površini preparata prominirali su lobuli tumorske mase koji su bili prekriveni poluprovidnom, sivoljubičastom, glatkom seroznom površinom, a sa jedne od resekcionih strana imali su neravnu i hrapavu saćastu građu (bez spoljne kapsule) (slika 2). Preseci tumora pokazivali su saćastu, delom sunđerastu sivobeličastu građu, sa najvećim brojem mikrocističnih promena prečnika 1–10 mm (slika 3). Mestimično uočavale su se male zone krvarenja ili hiperemije, a iz nekih cisti isticao je oskudni serozni sadržaj. Pojedine cistične šupljine bile su ispunjene koagulisanim i želatinoznim sadržajem. Dostavljena su ukupno četiri paketa limfnih žlezda, kao i materijal sa linije resekcije na holedohusu i telu pankreasa (radi biopsije *ex tempore*), kao i žučna kesa u kojoj je bilo nekoliko konktremenata manjih od 7 mm.

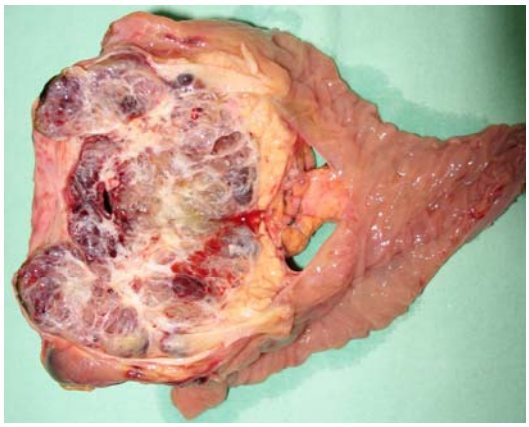
Mikroskopskim pregledom utvrđeno je da je, neoplastična proliferacija bila izgrađena od velikog broja mikrocista različite veličine, obloženih prostim kubičnim, mestimično aplatiranim epitelom bez nuklusne ili citološke atipije, ponegde sa pseudopapilarnim proliferatima (slika 4). Pojedine mikrociste pokazivale su prisustvo mrežastog koagulisanog proteinskog materijala, ali najveći broj mikrocisti bio je praznih lumena. Između njih, viđene su delimično kolagenizovane proširene fibrozne septe, koje su mestimično sadržavale dilatirane krvne sudove. U pojedinim područjima vaskularni prostori bili su umnoženi pseudo-hemangiomatoznom organizacijom.



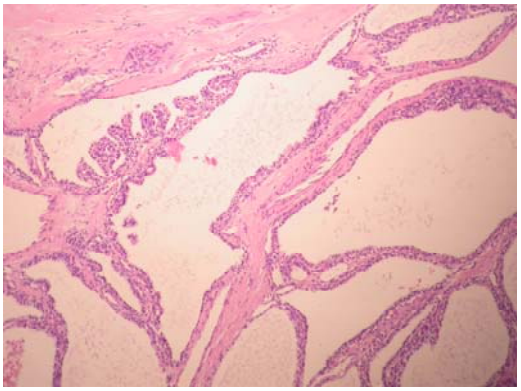
Sl. 2 – Recektovani preparat sa tumorom glave pankreasa

Bolesnica je operisana kroz obostranu supkostalnu laparotomiju. Nakon mobilizacije duodenuma i glave pankreasa viđen je polinodozni, policistični lividno plavičasti tumefakt, veličine muške pesnice, mekše konzistencije, sa više cisti ispunjenih bistrim tečnim sadržajem. Portna i gornja mezenterična vena bile su pritisnute tumorom uz izraženu stazu i dilataciju. Vrat pankreasa bio je sužen, širine do 1 cm, sa dila-

Histološkim pregledom utvrđeno je da je tumor bio seroznog, predominantno mikrocističnog tipa, mada su se ponegde uočavale i ciste veće od 10 mm. Iako nije imao kapsulu, tumor je bio jasno ograničen. Odnos prema okolini (ming) bio je ekspanzivan (Alpha). Tumor je ekspanzivno i po tipu pseudoinvazije rastao u okolno masno tkivo. Nije bilo invazije vaskularnih struktura. Linija resekcije holedo-



Sl. 3 – Na preseku tumora vidi se veliki broj cisti malih dimenzija



Sl. 4 – Mnogobrojne ciste različitih veličina obložene su regularnim kubičnim epitelom svetle citoplazme, koji fokalno grade mikropapile (HE; 64 ×)

husa bila je bez bitnih patohistoloških promena. Kao ni u tumoru, tako ni u jednoj žlezdi nisu postojale bilo kakve promene koje su mogle ukazati na malignitet. Druge patološke promene nisu uočene ni u pankreasu niti u ostalim delovima duktusnog sistema i duodenuma. Analizirani deo Wirsungovog kanala pokazao je fibroinflamatornu infiltraciju i neuronalnu hiperplaziju, ali elemenata tumora nije bilo.

Definitivni patohistološki nalaz bio je *Adenoma serosum microcysticum pancreatis*. Na žučnoj kesi nađena je holesteroloza.

Postoperativni tok bio je potpuno uredan. U daljem toku bolesnica je bila bez tegoba. Osim lekova za hipertenziju, nije bilo potrebe za drugom terapijom.

Diskusija

Utvrđeno je da se SMAP najčešće javlja u 7. i 8. deceniji života (prosečno 66 godina, raspon 34–91 godina) [6], češće kod žena (2/3 obolelih su žene), i u distalnom delu pankreasa. Veličina tumora obično je 6–10 cm (raspon 1–25 cm). Retko se javlja kod osoba sa von Hippel-Lindau sindromom [7]. Kod najvećeg broja obolelih tumor je loptast, dobro demarkiran, tj. ograničen, na preseku sunđeraste građe, često sa centralnim ožiljkom [2]. Izgrađen je od kuboidnih ćelija, porekla pankreasnih duktusa koje imaju bledu, glikogenom bogatu citoplazmu i malo, centralno postavljeno jedro [4]. Obično ostaje benigan [2]. Opisano je samo nekoliko slučajeva bolesnika sa malignom alteracijom SMAP [8–10]. Boje se PAS +.

Po pravilu, radi se o jednom SMAP, ali su, mada retko, opisivane i multifokalne forme tumora [3]. Retko se javlja u oligocističnoj pa i u unicističnoj formi, sa solidnom varijantom SMAPa [4, 11–13], a veoma retko udružen sa centralno lociranom endokrinom komponentom i to svih pet opisanih kod bolesnica koje su u proseku bile nešto mlađe nego bolesnice sa izolovanim SMAP [14, 15].

Imunohistohemijski, pozitivni su za citokeratin 7, 8, 18 i 19, a fokalno mogu biti pozitivni sa CA 19-9, B 72,3 dok su negativni na karcinoembrionalni antigen (CEA), tripsin, hromogranin A, sinaptofizin, S-100 protein, dezmin, vimentin i faktor VIII antigen.

Mada se US češće koristi pri traženju uzroka abdominalnog bola i nauzeje, KT je superioran u otkrivanju i karakterizaciji SMAP [5]. Na US vidi se grozdasta lobulirana lezija, koja, zbog velikog broja pregrada, može biti ehogena i solidna [5].

U diferencijalnoj dijagnozi treba uzeti u razmatranje razne lezije, mucinozne cistadenome, hemangiom, cistadenokarcinome acinarnih ćelija, serozne cistadenokarcinome i limfangiome. Neki autori uvereni su u značajnu dijagnostičku vrednost biopsije finom iglom (FNB), dok drugi upozoravaju da tek dobro poznavanje morfološkog spektra, korišćenje dodatnih analiza i korelacija sa kliničkim i radiološkim nalazima može značajno da poveća dijagnostičku vrednost FNB [16, 17].

Zaključak

S obzirom na nizak maligni potencijal SMAP, hirurški tretman, kao jedini koji može dovesti do izlečenja, nije neophodan kod svih bolesnika. Kada se radi o simptomatskim tumorima i kad je dijagnoza nesigurna, jedino hirurška ekscizija do u zdravo tkivo je metoda izbora kod ovih bolesnika, a obim i vrsta operacije zavise od lokalizacije i veličine tumora.

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Giant paraovarian cyst in a child complicated with torsion

Džinovska paraovarijumska cista komplikovana torzijom kod devojčice

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Abstract

Background. A variety of benign cyst may occur in and around the ovary and broad ligament and may simulate serous cystadenomas. The majority of broad ligament and paraovarian epithelial tumors are serous neoplasms of low malignant potential and presented with a pelvic mass with or without ascites or pain, but without involvement of the ovary. Ovarian torsion and paraovarian serous cystadenoma are rarely reported. **Case report.** We presented a case of giant paraovarian cyst in an 14-year-old girl, with characteristics of serous cystadenomas grossly and microscopically, and complicated with double adnexal torsion. A computed tomography scan showed large hypodense cystic mass (measuring 30 × 26 × 12 cm), occupying the whole abdominal cavity, with no adhesion to the surrounding organs. **Conclusion.** Precise clinical data as well as pathological examinations based on immunohistochemical stainings were important in making the diagnosis. These rare cystic lesions of para/mesoovarian location in children and their unclear histogenesis might be a histopathological diagnostical problem.

Key words:
ovarian cyst; cystadenoma; torsion; diagnosis; immunohistochemistry; adolescent.

Apstrakt

Uvod. Razne vrste benignih cisti mogu se naći unutar i oko jajnika i širokih ligamenata simulirajući serozne cistadenome. Većinu epitelnih tumora širokih ligamenata i okoline jajnika čine serozne neoplazme niskog malignog potencijala koje se prezentuju kao karlične mase sa ili bez ascitesa i bola, ali bez zahvatanja jajnika. Ovarijumska torzija i paraovarijumski serozni cistadenom retko su saopštavani. **Prikaz bolesnika.** Prikazali smo devojčicu od 14 godina sa džinovskom paraovarijskom cistom koja je imala makroskopske i mikroskopske odlike seroznog cistodenoma i bila komplikovana dvostrukom torzijom jajnika. Kompjuterizovana tomografija abdomena pokazala je veliku ovalnu hipodenznu cističnu formaciju dimenzija 30 × 26 × 12 cm, koja je ispunjavala čitavu trbušnu duplju, bez srastanja sa okolnim organima. **Zaključak.** Precizni klinički podaci, kao i patološka ispitivanja bazirana na imunohistohemijskim analizama značajna su u postavljanju dijagnoze. Ove, kod dece retke cistične lezije para/mezoovarijumske lokalizacije, kao i njihova nejasna histogeneza mogu biti patohistološki dijagnostički problem.

Ključne reči:
jajnik, cista; cistadenom; torzija; dijagnoza; imunohistohemija; adolescenti.

Introduction

A variety of benign cyst may occur in and around the ovary and broad ligament and simulate serous cystadenomas both grossly and microscopically [1]. Cystic lesions of the ovary are most common during infacy and adolescence, which are hormonally active periods of development [2]. Cysts are mostly nonneoplastic in children and could be categorized as follicular, simple, and corpus luteum cysts [3]. The rete ovarii rarely give rise to cysts and to benign and malignant tumors. These are most often found in postmenopausal women and only rarely in children [4–6]. The most common clinical presentation of ovarian cysts are abdominal pain, nausea and vomiting, and a history of previous episodes of similar pain and low grade fever [7].

One of the most intriguing aspects of ovarian epithelial neoplasms is their histogenesis. A suggestion is made that components of the secundary Müllerian system, which include paraovarian/paratubal cysts, rete ovarii, endosalpingiosis, endometriosis, and endomucinosis, merit some consideration as to their possible role in ovarian tumorigenesis [8].

We presented a case of giant paraovarian cyst in a 14-year-old girl, with characteristics of serous cystadenomas both grossly and microscopically, and complicated with double ovarian torsion.

Case report

Clinical data

A 14-year-old girl (strongly obese), was presented to the Department of Surgery, Military Hospital Niš, with lower

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right quadrant abdominal pain for the previous 24 hours, of moderate intensity and periodical characters, and not accompanied by nausea and vomiting.

Computed tomography (CT) showed a large hypodense cystic mass (measuring 30 × 26 × 12 cm), occupying the whole abdominal cavity (Figure 1). Laboratory analysis showed increased erythrocyte sedimentation rate – 32 mm/h (one hour), mild neutrophilia (white blood cell – WBC : 11.8 × 10^9/l; neutrophil leucocytes 83.4%). There were also signs of mild anemia syndrome with serum ferum/iron level decrease of 6.6 nmol/l. The remaining biochemical analyses showed normal values.

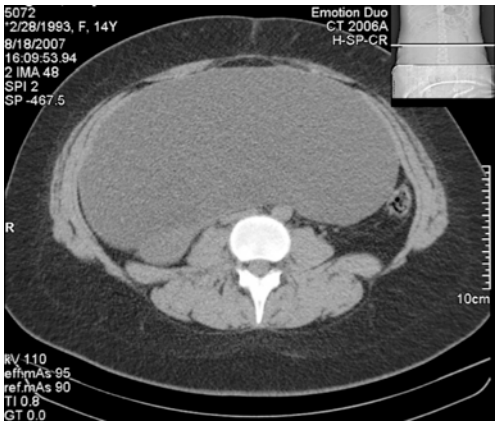

Fig. 1 – A computed tomography scan of the abdomen shows the hypodense cystic mass occupying the whole abdominal cavity

At laparotomy, a smooth cystic mass that originated from the right paraovarian tissues and extended to the upper abdomen was found, but without to taking the right ovary. Double right adnexal torsion was found, too. There was no adhesion to the surrounding abdominal organs. Aspiration from cyst was evacuated around 6 l of clear serous fluid. Complete excision of the cyst with the hemorrhagic infarcted right ovary was performed. Contralaterally, there was paraovarian serous cyst (measuring 7 × 4 × 4 cm), thin-walled, translucent and filled with clear watery fluid. Complete excision of the cyst was performed, and left adnexa was conservated. Pathologic examinations of the excised surgical material were performed.

Pathological findings

Giant empty cystic tumor had following characterictics: 15 cm in greatest diameter, smooth and wrinkled, glistening, white- greyish the external surface (Figure 2). On section, the unilocular cyst showed ragged and wrinkled greyish inner surface (Figure 3). The wall of the cyst was thicked and toughed, in some place separated and with hemorrhages.

Formalin-fixed, paraffin-embedded tissues samples were sectioned at 5 μm thick sections and stained with hematoxylin and eosin (HE), Alcian Blue – Periodic Acid Schiff (AB-PAS) and Masson Trichrome. Representative materials were stained with a panel of antibodies using the labeled streptavidin-biotin-peroxidase method according to the manufacturer's instructions (LSAB2 Kit, Dako). The primary antibodies used included estrogen receptor (ER) (clone 1D5), progesterone receptor (PR) (clone PgR636), vimentin (VIM) (clone V9), cytokeratin (CK) (clone AE1/AE3) and smooth muscle actin (SMA) (clone 1A4). The chromagen was 3,3'-diaminobenzidine (DAB), and the slides were lightly counterstained with Meyer's hematoxylin. All reagents were from Dako Company (Denmark, Copenhagen).

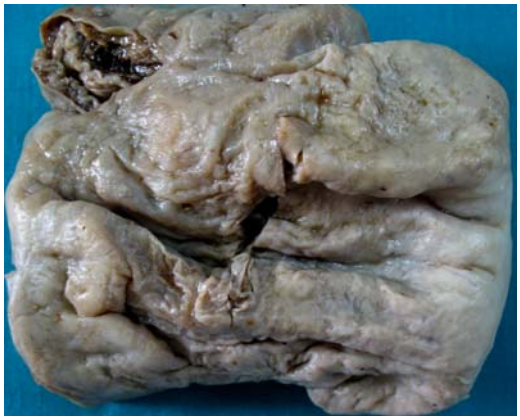

Fig. 2 – Gross specimen of large paraovarian cystic tumor, smooth and wrinkled, glistening, white-greyish the external surface

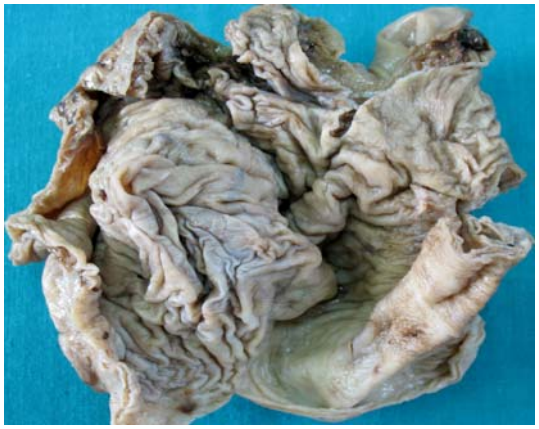

Fig. 3 – On section, unilocular cyst showed ragged and wrinkled greyish inner surface

Microscopically, the cyst wall was composed of fibro vascular tissue containing bundles of smooth muscle (Figure 4). The lining of cyst was composed of a single layer of tubal-type columnar epithelium with ciliae (Figure 5). A single layer of tubal-type columnar epithelium was strong immunoreactive for PR (Figure 6), VIM and CK. Immunoreactivity for ER was negative.

In the mesoovarian region some neurovascular elements were noted, while the hilus cells were not found despite the multiple analysis of several tissue samples. Microscopically, cystic and rare atretic folicules were discovered inside the membrane of the ovarian tissue sample, as well as rare corpus luteum in regression and morphological elements of hemorrhagic infarction.

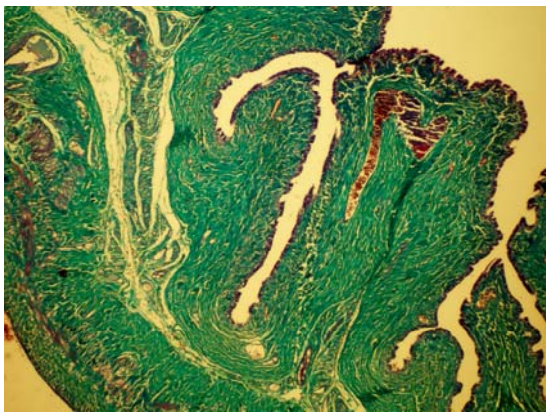

Fig. 4 – Cyst wall is composed of fibrovascular tissue containing bundles of smooth muscle (Masson trichrom, original magnification × 3.2)

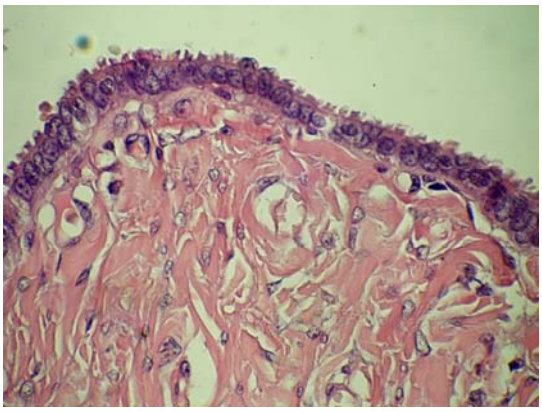

Fig. 5 – The lining of the cyst by tall columnar cells with prominent ciliae, without nuclear atypia, abnormal mitotic activity or pleomorphism (HE, original magnification × 40)

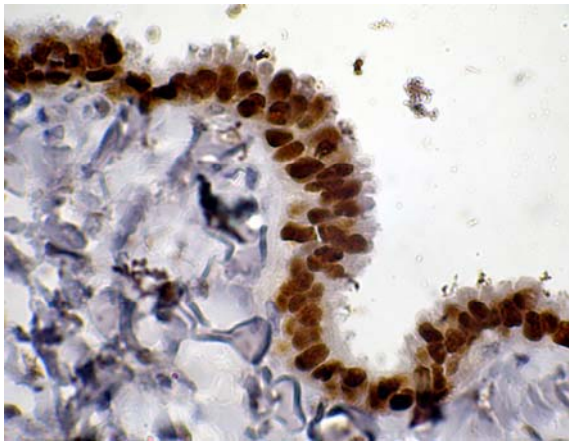

Fig. 6 – Tubal-type columnar epithelium show prominent nuclear staining for progesterone receptor (original magnification × 40)

Discussion

The majority of broad ligament and paraovarian epithelial tumors are serous neoplasms of low malignant potential and presented with a pelvic mass with or without ascites or pain, but without taking the ovary [9]. One of the most intriguing aspects of ovarian epithelial neoplasms is their histogenesis. The most common subtypes of these tumors are morphologically indistinguishable from neoplasm arising from those organs of the female genital tract that are embryologically derived from Müllerian ducts. Thus, the serous subtype is similar to tumors arising in the fallopian tubes. The currently favored hypothesis is that ovarian epithelial tumors arise from single cell layer lining the ovarian surface, which is often referred to as surface epithelium. This cell layer, which is continuous with the mesothelial lining of all pelvic and abdominal structures, is morphologically very similar to the mesothelial lining of peritoneal surface even away from the ovary. In addition, Müllerian-lined cysts are common not only in the ovary, and are also frequently seen in paraovarian tissues with no apparent direct connection to the ovary [8]. Laughlin used the term "secondary Müllerian system" to designate structures lined by Müllerian epithelium found oute of the uterus, cervix, and fallopian tubes [10].

A significant positive diffuse imune reactivity of the cystic epithelium for CK and VIM in this case can contribute to the suggested theory about mesothelial histogenesis of these tumors, and their Müllerian epithelium origin.

The rete ovary, the ovarian analogue of the rete testis, is a network of anastomosing tubules lined by flat, cuboidal, or columnar nonciliated cells with scanty eosinophilic or clear cytoplasm and located at the hilus of the ovary. Rete cysts are typically located in the ovarian hilus, most are unilocular, although occasionally they are multilocular [6, 11]. Rutgers and Scully [4] described 16 cases of rete cysts. In this series, the ages of the patients ranged from 23 to 80 years (mean, 59 years); all but 4 were postmenopausal. The cysts had a mean diameter of 8.7 cm (range 1–4 cm). In addition to their hilar location, clues to the origin of the cysts are an irregular contour of their inner surface with small crevice-like outpouchings and a wall that often contains bundles of smooth muscle and hyperplastic hilus cells [6].

The rete epithelium is immunoreactive for CK, VIM, and desmoplakin, as well as low levels of ER and PR [6, 8, 11].

The most common clinical presentations of ovarian cysts are abdominal pain, nausea and vomiting, and a history of previous episodes of similar pain and low grade fever [7].

Sudden pain and mild hyperthermia were the clinical manifestations in our case, but without ascites. After the evacuation of 6 l of serous fluid, the paraovarian location of the cyst was clearly distinguished from the surrounding organs and the ovary itself.

Because infundibulopelvic pedicle is longer in a child, torsion is a significant risk for larger cysts. Ovarian torsions are rare in the pediatric age group. In addition, atrophy of the ovary and other complications are common [2, 6, 7, 12]. In our case, the giant paraovarian cyst was accompanied by some complications including a double torsion, and initial hemorrhagic infarction of the ovary in question.

Conclusion

Our case report shows that the ovarian epithelial cystic neoplasm of paraovarian location may develop in children.

The cystic neoplasm of paraovarian location may reach a large size and cause numerous complications, the most frequent of whicn is adnexal torsion, which could be prevented by an early diagnosis and surgical treatment. These rare cystic lesions of paraovarian/mesoovarian location in children and their unclear histogenesis might be a histopathological diagnostic problem. According to our opinion, in making the diagnosis of these cystic neoplasms it is necessary to make (immuno) histochemical analyses on big series of tissue samples, as well as to know precise clinical data.

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P R I K A Z S L U Č A J A UDC: 616.12-008.313:616.126-007.1

Epštajnova anomalija kao uzrok paroksizmalne atrijalne fibrilacije

Ebstein's anomaly as a cause of paroxysmal atrial fibrillation

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Apstrakt

Uvod. Epštajnova anomalija karakteriše se pomeranjem trikuspidne valvule ka apeksu zbog anomalnog pripoja trikuspidnih kuspisa. Kod više od polovine bolesnika postoje *Wolff-Parkinson-White* (WPW) sindrom i paroksizmalna aritmija. **Prikaz bolesnika.** Prikazana je žena, stara 32 godine, koja je imala česte paroksizme atrijalne fibrilacije. Posle konverzije ritma EKG je pokazivao WPW sindrom. Ehokardiografskim pregledom nađena je normalna veličina levih srčanih šupljina sa paradoksnim pokretima komorskog septuma. Desna komora bila je vrlo mala zbog njene atrijalizacije. Početak trikuspidne valvule bio je 20 mm bliži apeksu desne komore nego početak mitralne valvule. Elektrofiziološkim ispitivanjem dokazano je prisustvo desnog posterolateralnog akcesornog puta. Atrijalna fibrilacija lako je izazvana u elektrofiziološkoj laboratoriji, a zatim je učinjena uspešna ablacija akcesornog puta. Posle toga nije bilo WPW sindroma ni paroksizama atrijalne fibrilacije. **Zaključak.** Epštajnova anomalija jedan je od razloga paroksizmalne atrijalne fibrilacije, naročito kod mladih osoba sa WPW sindromom.

Ključne reči:
anomalija, epštajnova; dijagnoza; elektrokardiografija; ehokardiografija; fibrilacija pretkomora; sindrom, wolff-parkinson-white.

Abstract

Background. Ebstein´s anomaly is characterized by a displacement of the tricuspid valve toward apex, because of anomalous attachment of the tricuspid leaflets. There are type B of Wolff-Parkinson-White (WPW) syndrome and paroxysmal arrhythmias in more than a half of all patients. **Case report.** We presented a female, 32-year old, with frequent paroxysms of atrial fibrillation. After conversion of rhythm an ECG showed WPW syndrome. Echocardiographic examination discovered normal size of the left cardiac chambers with paradoxical ventricular septal motion. The right ventricle was very small because of its atrialization. The origin of the tricuspid valve was 20 mm closer to apex of the right ventricle than the origin of the mitral valve. Electrophysiological examination showed a posterolateral right accesorial pathway. Atrial fibrillation was induced very easily in electrophysiological laboratory and a successful ablation of accessorial pathway was made. There were no WPW syndrome and paroxysms of atrial fibrillation after that. **Conclusion.** Ebstein´s anomaly is one of the reasons of paroxysmal atrial fibrillation, especially in young persons with WPW syndrome.

Key words:
ebstein's anomaly; diagnosis; electrocardiography; echocardiography; atrial fibrillation; wolff-parkinson-white syndrome.

Uvod

Epštajnova anomalija (EA) retko je kongenitalno srčano oboljenje koje čini 0,3–0,8% svih urođenih srčanih mana. Javlja se kod 1–5 na 200 000 živorođenih [1]. Učestalost se ne razlikuje među polovima.

Anomalija se odlikuje pomeranjem trikuspidne valvule ka apeksu, zbog anomalnog pripoja trikuspidnih kuspisa. Kod 14–20% bolesnika sa EA postoji *Wolff-Parkinson-White* (WPW) sindrom, češće tip B, i jedan ili više akcesornih puteva, većinom lokalizovanih oko izmenjene trikuspidne valvule pri čemu su skoro svi na desnoj strani srca (slobodni zid ili septa), dok se levostrani akcesorni putevi nalaze kod samo 3,8% bolesnika [2, 3]. Sindrom WPW viđa se i kod drugih urođenih srčanih oboljenja, ali 1/3 svih bolesnika sa urođenim srčanim manama i WPW sindromom ima EA. Oko 25% bolesnika sa EA ima epizode paroksizmalne atrijalne tahikardije [4]. Atrijalna fibrilacija obično se javlja posle 20. godine života.

Prikaz bolesnika

Žena, u dobi od 32 godine, javila se u prijemnu ambulantu zbog gušenja pri naporu i zamora. Slične tegobe imala je tokom poslednjih godinu dana. Nekoliko puta ambulantno

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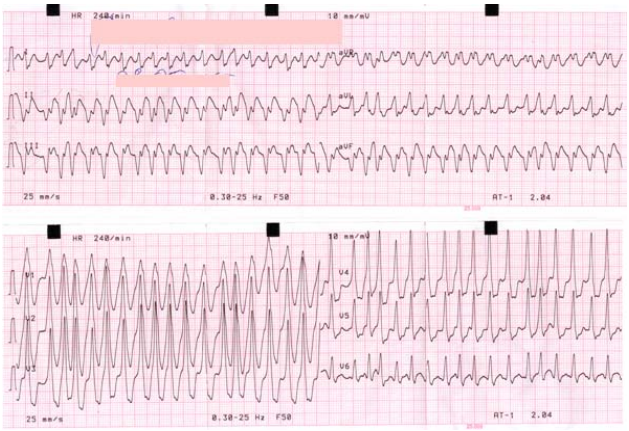
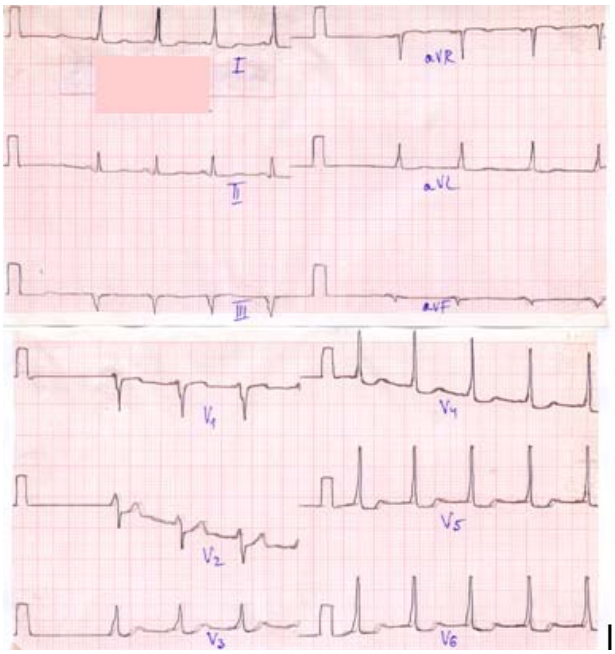
joj je rađena konverzija ritma, ali nije sprovedeno kardiološko ispitivanje. Auskultatorni nalaz na plućima bio je normalan, a auskultacijom srca otkriven je sistolni šum trikuspidne regurgitacije nad donjom polovinom sternuma. Krvni pritisak iznosio je 105/70 mmHg, srčana frekvencija oko 200/min. Nije bilo hepatomegalije ni otoka potkolenica. Laboratorijske analize bile su u granicama normalnih vrednosti. EKG je pokazivao paroksizam atrijalne fibrilacije sa širokim QRS kompleksima (slika 1).



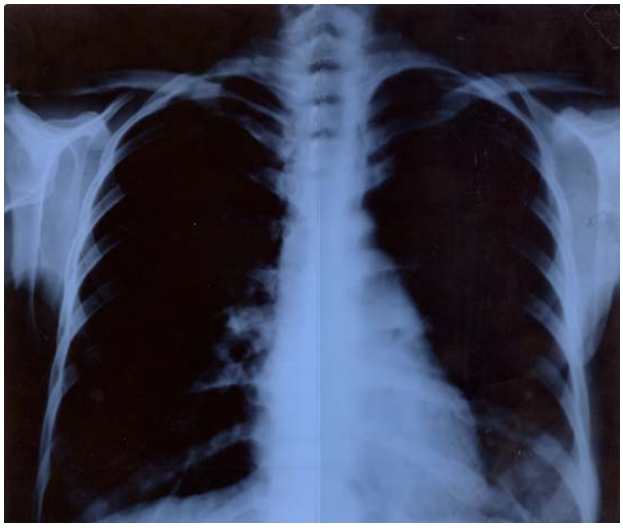
Sl. 1 – EKG pokazuje paroksizam atrijalne fibrilacije sa širokim QRS kompleksima

Posle medikamentne konverzije infuzijom amjodarona EKG je pokazao sindrom preekscitacije tipa B (slika 2).



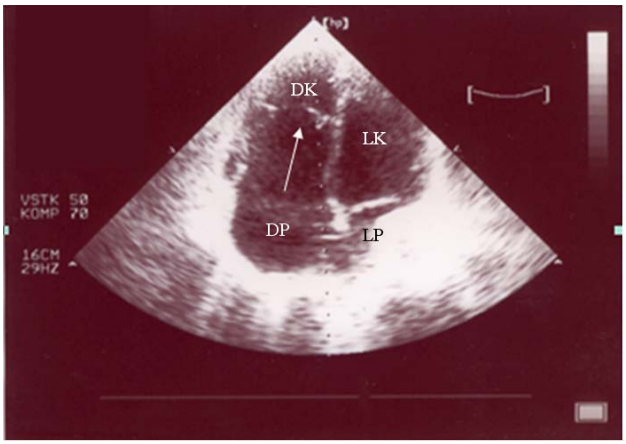
Sl. 2 – EKG bolesnice sa EA pokazuje kratak PQ interval sa tipom B sindroma preekscitacije

Rendgenogram grudnog koša pokazao je srčanu siluetu normalne veličine i ispunjen plućni zaliv. Kardiotoraksni odnos iznosio je 0,44 (slika 3).



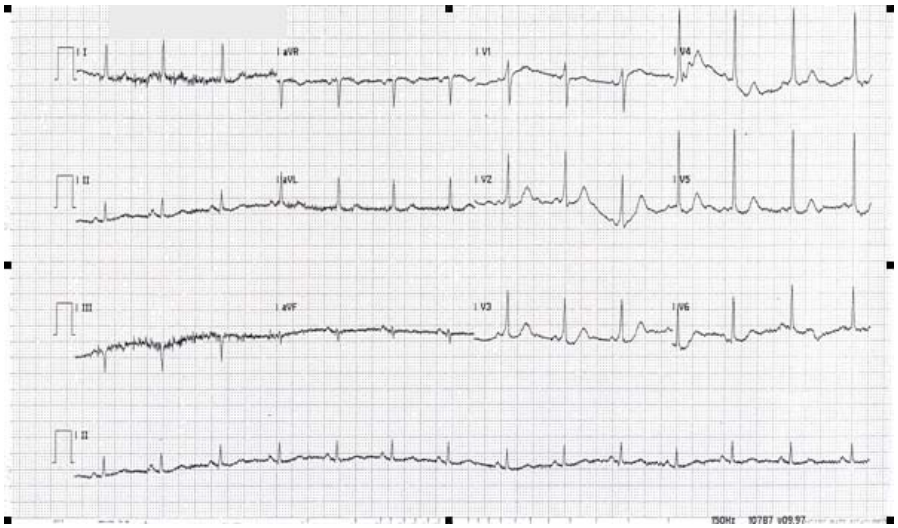
Sl. 3 – Rendgenogram srca pokazuje srčanu siluetu normalne veličine i ispunjen plućni zaliv

Ehokardiografskim pregledom nađena je normalna veličina levih srčanih šupljina sa paradoksnim pokretima komorskog septuma. Ejekciona frakcija leve komore iznosila je 60%. Desna komora bila je vrlo mala zbog njene atrijalizacije. Desna pretkomora bila je uvećana (44 mm, mereno iz apeksnog preseka četiri šupljine). Početak trikuspidne valvule bio je 20 mm bliži apeksu desne komore nego početak mitralne valvule. Doplerom je nađen trag trikuspidne regurgitacije sa sistolnim pritiskom u desnoj komori od 30 mmHg. Nije uočena komunikacija na nivou pretkomora niti između komora (slika 4).



Sl. 4 – Apeksni presek 4 šupljine pokazuje apeksno pomeranje septalnog kuspisa triksupidne valvule (strelica).
Desna pretkomora (DP) izrazito je uvećana (DK=desna komora, LP=leva pretkomora, LK=leva komora)

Elektrofiziološko ispitivanje pokazalo je postojanje desnog posterolateralnog akcesornog puta. Atrijalna fibrilacija lako je izazvana u elektrofiziološkoj laboratoriji, a onda je učinjena uspešna ablacija akcesornog puta. Posle toga nije bilo preekscitacionog sindroma i atrijalne fibrilacije (slika 5).



Sl. 5 – EKG bolesnice posle uspešne ablacije akcesornog puta

Diskusija

Dramatičan napredak u dijagnostici i terapiji EA započeo je njenim prvim opisom, 1866. godine. Ta anomalija danas se najlakše dijagnostikuje ehokardiografskim pregledom. U prošlosti dijagnoza je postavljana najčešće na autopsiji. Do 1950. godine opisana su samo tri bolesnika sa EA u prvom volumenu časopisa Circulation.

Najčešći inicijalni simptomi EA u detinjstvu su cijanoza, loša tolerancija napora i aritmije. Kardiomegalija i sistolni šum su karakterističan nalaz, a od aritmija nalaze se paroksizmalna supraventrikularna tahikardija ili atrijalna fibrilacija ili flater. Česta ektopična električna aktivnost može biti posledica istezanja zida velike desne pretkomore, što značajno povećava mortalitet operisanih bolesnika [5].

Bolesnica je više puta imala kraće ili duže napade lupanja srca, bez težih simptoma. Uglavnom se sve završavalo na medikamentnoj konverziji ritma u ambulantnim uslovima. Tek nakon urađenog ehokardiografskog pregleda postavljena je dijagnoza EA, a potom je urađeno i elektrofiziološko ispitivanje. Posle ablacije akcesornog puta bolesnica nije imala paroksizme atrijalne fibrilacije. Ovaj primer pokazuje da u sličnim prilikama obavezno treba uraditi ehokardiografski pregled, a ne samo jednostavno usmeriti pažnju na konverziju u sinusni ritam.

Khositseth i sar. [6] ispitivali su 130 bolesnika sa EA od kojih je 109 imalo aritmije, zbog čega su podvrgnuti elektrofiziološkom ispitivanju. Njih 48 (44%) hirurški je lečeno zbog atrijalnog flatera ili fibrilacije. Posle skoro tri godine praćenja 75% ovih bolesnika nije imalo aritmije.

Naprasna smrt sreće se kod 3–10% bolesnika sa EA. Smatra se da ona nastaje sekundarno zbog brzog sprovođenja supraventrikularne tahikardije ili atrijalne fibrilacije ili flatera u komore. Kod 6% bolesnika sa urođenim srčanim manama uzrok naprasne smrti je EA, što je veliki procenat za tako retku anomaliju. Smrtnost u svim starosnim grupama iznosi 12,5%.

Za bolesnike bez simptoma, kao i bolesnike sa simptomima, ali bez desno-levog šanta i sa blagom kardiomegalijom, preporučuje se samo praćenje. Deca koja prežive najranije detinjstvo uglavnom se dobro osećaju dugi niz godina. Bolesnike sa tahiaritmijom koji ne reaguju na medikamentnu terapiju treba tretirati radiofrekventnom ablacijom čija je stopa uspeha 90–100% [2]. Operativno lečenje može se odložiti dok se ne pojave ili pojačaju simptomi, izražena cijanoza, paradoksne embolije, policitemija, refraktarne aritmije ili kardiotoraksni odnos koji je jednak 0,65 ili veći i koji je bolji prediktor naprasne smrti nego funkcionalni status bolesnika [5].

Hirurško lečenje dolazi u obzir kada postoje objektivni dokazi pogoršanja bolesti kao što su povećanje srčane siluete na rendgenogramu srca, progresivna dilatacija desne komore ili smanjenje sistolne funkcije na ehokardiografskom pregledu ili kada se pojave komorske ekstrasistole ili atrijalne tahiaritmije. Kada simptomi progrediraju do New York Heart Association (NYHA) klase III ili IV, hirurško lečenje je jasno indikovano [1].

Prva uspešna hirurška intervencija zbog trikuspidne regurgitacije kod bolesnika sa EA opisana je 1962. godine, kada je trikuspidna valvula zamenjena protezom [1]. Različite hirurške tehnike se koriste za prevazilaženje hemodinamskih posledica EA, kao što su rekonstrukcija ili zamena abnormalne trikuspidne valvule, plikacija atrijalne osnove ili samo zatvaranje atrijalne komunikacije, ukoliko ona postoji. Resekcija desne pretkomore smanjuje njene dimenzije i istezanje njenog zida. Biventrikularna rekonstrukcija obično je moguća, ali ako nastane uznapredovala kardiomiopatija, naročito ako zahvata levu komoru, onda je transplantacija srca jedino rešenje [1, 5].

Profilaksa infektivnog endokarditisa preporučuje se kod bolesnika sa EA, mada je rizik od njegove pojave mali. Suprotno, rizik od naprasne smrti ostaje značajan problem kod bolesnika sa EA bez obzira na težinu bolesti ili način lečenja [5].

Postoji značajno smanjenje nastanka atrijalne fibrilacije posle hirurške ablacije akcesornih puteva kod bolesnika sa EA, bez obzira da li je istovremeno rađena i huirurška korekcija same EA. To ukazuje da etiologija atrijalne fibrilacije

kod mnogih od ovih bolesnika verovatnije može biti povezana sa prisustvom akcesornih puteva nego sa primarnim ili sekundarnim anbormalnostima atrijalnog miokarda koje postoje u EA. Mada ablacija akcesornog puta (puteva) dugotrajno smanjuje postoperativnu učestalost atrijalne fibrilacije kod bolesnika sa EA, atrijalna fibrilacija ipak još uvek ima tendenciju javljanja 2–3 puta češće kod tih bolesnika nego kod osoba bez srčanog oboljenja [3, 7].

Epštajnova anomalija je kongenitalno oboljenje koje nije ograničeno samo na desno srce. Anomalije levog srca često se opisuju kod bolesnika sa EA i uključuju primarno disfunkciju leve komore zbog paradoksnih pokreta komorskog septuma, starosti bolesnika, prisustva bolesti mitralne valvule, endokardne fibroze i retko miokardnog infarkta. Abnormalnosti šupljine leve komore, njene konture i kontraktilnosti opisane su kod 67% bolesnika. Attenhofer Jost i sar. [8] opisali su tri bolesnika sa EA koji su imali ehokardiografske znake za *non-compaction* levu komoru. Jedan bolesnik imao je tešku biventrikularnu srčanu insuficijenciju koja je zahtevala transpalantaciju srca. Abnormalnosti levog srca koje uključuju *noncompacted* miokard, bikuspidnu aortnu valvulu, komorski septalni defekt i oboljenja mitralne valvule viđaju se kod 39% bolesnika [8].

Prikazana bolesnica je bez tegoba i atrijalnih aritmija i posle četiri godine nakon uspešne radiofrekventne ablacije akcesornog puta.

Zaključak

Epštajnova anomalija jedan je od uzroka atrijalnih aritmija, pa i atrijalne fibrilacije, naročito kod mladih osoba sa sindromom preekscitacije. Prognoza EA je različita i zavisi uglavnom od stepena cijanoze i funkcije trikuspidne valvule, na koju utiče atrijalni šant (ako je prisutan). Učestalost aritmija nezavisan je faktor. Zbog toga, svim bolesnicima sa EA obavezno treba uraditi ehokardiografski pregled kojim se relativno lako otkriva ova urođena anomalija i procenjuje funkcija triksupidne valvule, a zatim i elektrofiziološko ispitivanje kojim se otkriva prisustvo i lokalizacija akcesornih puteva i time omogućuje adekvatno lečenje.

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P R I K A Z S L U Č A J A UDC: 616.126.42-005.6:615.273.015.8

Neokluzivna tromboza veštačke mitralne valvule kod neadekvatno zbrinute bolesnice sa rezistencijom na antikoagulantnu terapiju

Nonoclusive thrombosis of mechanical mitral valve prosthesis caused by inadequate treatment of anticoagulant therapy resistance

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Apstrakt

Uvod. Oralna antikoagulantna sredstva koriste se duže od šest decenija u prevenciji tromboembolijskih komplikacija. Redak ali moguć problem kod primene tih lekova može predstavljati rezistencija na njih. **Prikaz bolesnika.** Prikazana je neadekvatno lečena bolesnica sa rezistencijom na peroralnu antikoagulantnu terapiju, kod koje je došlo do neokluzivne tromboze mehaničke mitralne proteze. Rezistencija na oralne antikoagulantne lekove kod nje je dokazana na osnovu toga što povećavanje doze varfarina do 20 mg, a zatim i acenokumarola na 15 mg tokom deset dana nije dovelo do povećanja vrednosti *international normalised ratio* (INR) preko 1,2. Na osnovu podataka da nije uzimala hranu bogatu K vitaminom, ni lekove koji bi mogli umanjiti dejstvo oralnih antikoagulantnih lekova, kao i da nije imala pridružene bolesti i stanja koja bi bila uzrok neadekvatnog odgovora na antikoagulantnu terapiju, posredno je zaključeno da se radi o hereditarnom obliku rezistencije. Zbog postojeće mehaničke proteze na mitralnoj poziciji u terapiju je uveden niskomolekulski heparin čiju je dozu samoinicijativno smanjila, što je sve dovelo do neokluzivne tromboze valvule. **Zaključak.** Kada ne postoje pridružene komplikacije kao što je apsolutna aritmija, nalaz rezistencije na oralna atikoagulantna sredstva indikacija je za zamenu mehaničke proteze biološkom, što je i učinjeno kod prikazane bolesnice.

Ključne reči:
zalistak, mitralni, stenoza; zalisci srca, veštački; lečenje; antikoagulansi; lekovi, rezistencija; tromboza.

Abstract

Background. Oral anticoagulants have been used in the prevention of thromboembolic complications for over six decades. A rare, but possible problem in the application of these medications could be resistance to them. **Case report.** We presented a patient with nonocclusive thrombosis of the mechanical mitral prosthesis due to inadequately treated resistance to peroral anticoagulant therapy. Resistance to oral anticoagulant medications was proven by an increased dosage of warfarin up to 20 mg and, after that, acenokumarol to 15 mg over ten days which did not lead to an increase in the international normalised ratio (INR) value over 1.2. On the basis of information that she did not take food rich in vitamin K or medications which could reduce effects of oral anticoagulants, and that she did not have additional illnesses and conditions that could cause an inadequate response to anticoagulant therapy, it was circumstantially concluded that this was a hereditary form of resistance. Because of the existing mechanical prosthetics on the mitral position, low molecular heparin has been introduced into the therapy. The patient reduced it on her own initiative, leading to nonocclusive valvular thrombosis. **Conclusion.** When associated complications like absolute arrhithmia does not exist, the finding of resistance to oral anticoagulant agents is an indication for the replacement of a mechanical prosthetic with a biological one which has been done in this patients.

Key words:
mitral valve stenosis; heart valve prosthesis; therapeutics; anticoagulants; drug resistance; thrombosis.

Uvod

Kod bolesnika sa mehaničkim valvulama postoji visok rizik od tromboze valvule i posledične sistemske embolizacije. Neorganski materijali od kojih su proteze sačinjene i nefiziološki protok krvi oko njih predstavljaju razloge za visok tromboembolijski potencijal mehaničkih valvula. Prevencija ove komplikacije svodi se na primenu peroralne antikoagulantne terapije. Uprkos redovnoj primeni antikoagulantne terapije incidencija tromboembolijskih komplikacija iznosi 1–2% godišnje, a ukoliko se antikoagulantna terapija ne koristi ova incidencija se povećava na 10% godišnje [1]. Pored neadekvatne primene peroralne antikoagulantne terapije, zbog nemara i neprosvećenosti i interakcija sa nekim leko-

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vima i hranom, kao mogući uzrok tromboembolijskih komplikacija kod bolesnika sa mehaničkim valvulama, pominje se i rezistencija na antikoagulantnu terapiju.

Prikazana je neadekvatno lečena bolesnica sa rezistencijom na peroralnu antikoagulantnu terapiju kod koje je došlo do neokluzivne tromboze mehaničke mitralne proteze.

Prikaz slučaja

Bolesnica, u dobi od 44 godine, preležala je u osmoj godini reumatsku groznicu, a sedam godina kasnije saznala za srčanu manu – mitralnu stenozu sa mitralnom regurgitacijom. U 39. godini života pojavili su se simptomi levostrane srčane insuficijencije zbog čega joj je četiri godine kasnije učinjena zamena mitralne valvule *Medtronic Hall* protezom N25. Međutim, redovnim kontrolama protorombinskog vremena i vrednosti *International normalised ratio* (INR), koji predstavlja odnos protrombinskog vremena bolesnika i kontrolnog protrombinskog vremena, utvrđeno je da se ne ostvaruje adekvatna korekcija neophodna za prisustvo veštačke valvule. Indirektno, dokazano je da postoji rezistencija na peroralna antikoagulantna sredstva. Naime, povećavanje doze varfarina do 20 mg, a zatim i acenokumarola na 15 mg tokom deset dana nije dovelo do povećanja vrednosti INR preko 1,2. Posredno, zaključeno je da postoji rezistencija na ove lekove. U terapiju je uveden niskomolekulski heparin, čiju je dozu bolesnica samoinicijativno menjala. Međutim, kod bolesnice su se nakon osam meseci pojavile tegobe – bolovi u grudima promenljivih karakteristika, intenziteta i trajanja zbog kojih je hospitalizovana.

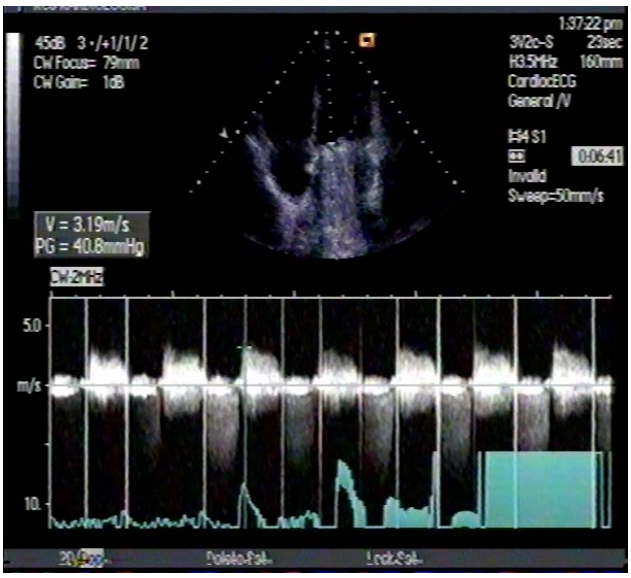
Objektivnim pregledom pri prijemu nađeno je da je bolesnica bila svesna, bez povišene telesne temperature. Nisu postojali znaci periferne limfadenopatije. Vene vrata bile su neupadljive. Nad plućima je postojao normalan disajni šum, a na srcu pravilna srčana radnja, čujan zvek veštačke valvule i rani aspiracioni dijastolni šum uz levu ivicu grudne kosti (2–3/6), srčana frekvencija 90/min, krvni pritisak 140/90 mmHg. Jetra i slezina nisu se palpirale i nisu postojali pretibijalni edemi.

Laboratorijskim analizama nađeni su lako ubrzana sedimentacija (30 prvi sat), normalne vrednosti hemoglobina (124 g/l) i hematokrita (0,35), normalan broj leukocita ($6,3 \times 10^9$/l) i trombocita (233×10^9/l), veće vrednosti glukoze (10,6; 8,3; 4,3 mmol/l), normalne vrednosti uree (5,7 mmol/l), kreatinina (94 μmol/l), bilirubina (5,3 μmol/l), ukupnih proteina (65 g/l), natrijuma (143 mmol/l), kalijuma (4,1 mmol/l), transaminaza: serumska aspartat aminotransferaza (S-AST) (26 IJ/l), serumska alanin aminotransferaza (S-ALT) (16 IJ/l) i značajno veće vrednosti laktat dehidrogenaze (654 IJ/l). Nivoi faktora koagulacije bili su u okvirima referentnih vrednosti (faktor II 85%, faktor VII 94%).

Rendgenogramom grudnog koša nađena je srčana senka normalnog oblika, veličine i položaja, a u plućnom parenhimu nisu uočene aktivne patološke promene.

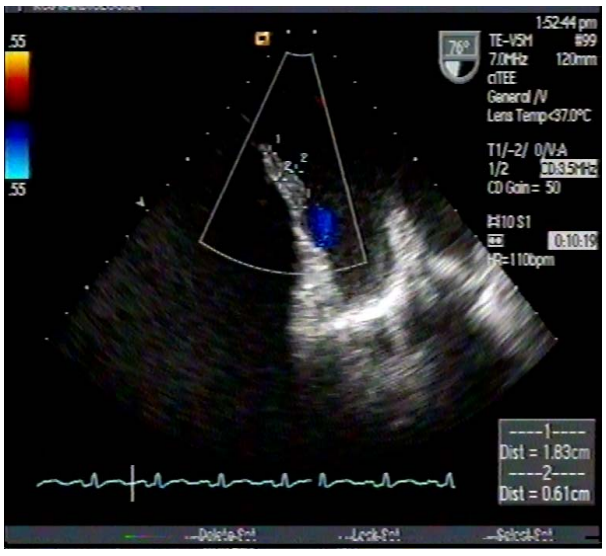
Prvi transtoraksni ehokardiografski nalaz ukazao je na postojanje neokluzivne tromboze mitralne proteze sa povećanjem gradijenta preko valvule (maksimalni gradijent iznosio je 31 mmHg, a srednji 15 mmHg), uz blagu transvalvularnu regurgitaciju. U izlaznom traktu leve komore, čiji promer je bio normalan, registrovana je umerena aortna regurgitacija.

Nakon tog ehokardiografskog pregleda uveden je heparin u kontinuiranoj intravenskoj infuziji. Dve nedelje kasnije, ponovnim transtoraksnim ehokardiografskim pregledom utvrđeno je da ne samo da se održava neokluzivna tromboza veštačke valvule plasirane na mitralnoj poziciji, već i da se maksimalni gradijent povećao i da iznosi 40 mmHg (slika 1).



Sl. 1 – Neokluzivna tromboza arteficijalne mitrale valvule

Učinjenim transezofagusnim pregledom nađeno je da se sa pretkomorske strane duž čitavog anulusa nalazi trombotska masa debljine 6–7 mm (slika 2). U levoj aurikuli nisu uočene trombotske mase već samo vrtloženje krvi. Kolor doplerom u levoj pretkomori registrovana je blaga transvalvularna mitralna regurgitacija. Istovremeno, nađeno je da je aortna valvula promenjena, sa aneurizmom jednog od Valsalvinih sinusa i umerenom aortnom regurgitacijom.



Sl. 2 – Trombotska masa duž mitralnog anulusa (TEE)

Zbog neosetljivosti na primenjenu antikoagulantnu terapiju zaključeno je da je potrebna hirurška reintervencija – zamena postojeće mehaničke proteze biološkom. Šest meseci nakon intervencije stanje bolesnice bilo je stabilno.

Diskusija

Rezistenciju na peroralna antikoagulantna sredstva često poistovećujemo sa rezistencijom na varfarin zato što je on najčešće propisivan oralni antikoagulans na svetu. Kao mogući razlog tog poistovećivanja je i to što varfarin, kao lek, služi za primer kako hereditet i faktori okruženja (ishrana, unos nekih lekova), demografski činioci i rasa utiču na razlike u interindividualnom odgovoru [2].

Značajna promenljivost individualnog odgovora ogleda se u činjenici da propisana doza varfarina varira od 1 do 40 mg [3]. Kod 95% bolesnika na oralnoj antikoagulantnoj terapiji zadovoljavajući terapijski odgovor postiže se dozom varfarina od 1 do 9 mg [4]. Na osnovu toga, Routledge i sar. [4] rezistenciju na varfarin definisali su kao potrebu za davanjem više od 9 mg dnevno. Međutim, definicija rezistencije trpela je izmene i danas je prihvaćeno da ona postoji kada je potrebno uneti više od 20 mg leka na dan [5]. Razlozi za rezistenciju na oralne antikoagulantne lekove mogu se podeliti na one koji utiču na farmakokinetiku i one koji menjaju njihovu farmakodinamiku [4, 6]. U farmakokinetske razloge za rezistenciju ubrajaju se oni koji utiču na apsorciju i eliminaciju lekova, a u farmakodinamske oni koji menjaju hemostatski odgovor na lek dat u određenoj dozi zbog uticaja na sintezu i klirens K vitamin-zavisnih faktora koagulacije. Uprkos činjenici da je ovom podelom jasno definisan mehanizam nastanka rezistencije na oralne antikoagulantne lekove, šire je prihvaćena pojednostavljena podela na stečenu i naslednu rezistenciju.

Stečena rezistencija definiše se kao rezistencija nastala zbog interakcije oralnih antikoagulantnih sredstava sa hranom i lekovima. Najčešći uzrok stečene rezistencije je unos hrane bogate vitaminom K. U našoj sredini luk, zeleno povrće i riba, uneti u većoj količini (više od 250 g/dan), mogu dovesti do kompeticije sa oralnim antikoagulansima na nivou hepatocita. U zemljama severne Amerike najviše se pominje interakcija sa avokadom i brokolima [7]. Parenteralna nutricija može biti uzrok stečene rezistencije na varfarin i to zbog vezivanja leka za proteinske komponente u njoj [8]. Pored poznatog povećanja senzitivnosti na varfarin, hronični unos alkohola, kod nekih bolesnika može potencirati klirens varfarina i na taj način usloviti potrebu za povećanjem doze leka [6].

Rezistencija na oralne antikoagulanse neizbežno se javlja kod bolesnika koji sedam dana unose više od 5 mg vitamina K [7]. Wells i sar. [9] su utvrdili da primena osam od 86 ispitanih lekova i supstancija dovodi do pojave rezistencije na varfarin. Od lekova koji inhibišu dejstvo varfarina najčešće se pominju holestiramin, barbiturati, hlordiazepoksid, nafcilin. Oni različitim mehanizmima utiču na metabolizam oralnih antikoagulantnih lekova. Holestiramin i holestipol smanjuju apsorciju varfarina. Barbiturati, karbamezapin i rifampicin indukcijom enzimskog sistema povećavaju eliminaciju oralnih antikoagulantnih sredstava. Estrogeni, grizeofulvin, 6-merkaptopurin, haloperidol umanjuju antikoagulantni efekat varfarina, ali mehanizam njihovog delovanja nije jasan. Opisani su pojedinačni slučajevi rezistencije zbog korišćenja sulfasalazina i teikoplanina [6, 10].

Pored unosa hrane, nekih supstancija i lekova koji utiču na individualnu osetljivost prema varfarinu, na njegovu primenu utiče i hipotireoza zbog smanjenog klirensa faktora koagulacije zavisnih od vitamina K koji umanjuju efikasnost varfarina. Mogući uzrok rezistencije na oralne antikoagulantne lekove je i malapsorcija [5]. Hiperholesterolemija može biti uzrok prolazne rezistencije na varfarin [4].

Kod prikazane bolesnice indirektno je isključeno postojanje stečene rezistencije. Naime, ona nije uzimala hranu bogatu vitaminom K, takođe nije uzimala nijedan od lekova koji bi mogao da umanji dejstvo oralnih antikoagulantnih lekova, a nije postojalo nijedno pridruženo oboljenje ili stanje koje bi uticalo na odgovor na oralnu antikoagulantnu terapiju. Koncentracija faktora koagulacije, takođe, bila je normalna. Nažalost, u periodu kad je prikazana bolesnica lečena nije bilo mogućnosti za određivanje koncentracije leka u krvi koja je inače visoka kod nasledne rezistencije. Tako, moglo se pretpostaviti da se radi o naslednoj rezistenciji na oralne antikoagulantne lekove.

Pre više od pet decenija započeto je sa primenom oralnih antikoagulantnih lekova prilikom uništavanja glodara. Pre više od tri decenije objavljeni su prvi prikazi genetski uslovljene senzitivnosti i rezistencije na varfarin [11]. Prvo je otkriven gen odgovoran za enzim CYP2C9 koji ima ulogu u metabolizmu farmakološki potentnijeg S-enantiomera varfarina u inaktivne metabolite i genetske varijacije koje u značajnoj meri smanjuju aktivnost enzima. Rasvetljena genetski uzrokovana senzitivnost na varfarin navela je na pretpostavku da je povećanje aktivnosti CYP2C9 odgovorno za farmakokinetski oblik hereditarne varfarinske rezistencije [12]. Međutim, uloga enzima CYP2C9 u klirensu varfarina nije dovoljno dokazana, ali utvrđeno je da na njega utiču genske duplikacije i mutacija enzima P450 u njegovom fenotipskom ispoljavanju [13].

Drugi, daleko značajniji farmakodinamski oblik hereditarne varfarinske rezistencije nastaje zbog mutacije gena za epoksid reduktazu vitamina K (VKOR) koji se kod ljudi nalazi na 16. hromozomu [14]. Poznato je da varfarin deluje inhibicijom enzima VKOR. Taj enzim reciklira vitamin K 2,3 epoksid u redukovani vitamin K koji se oksidacijom vraća u formu vitamin K hidrokinon koji je uključen u karboksilaciju i aktivaciju faktora koagulacije II,VII, IX i X. Rost i sar. [14] identifikovali su gen VKOR kompleks subjedinica 1 (VCORC1) sa glavnom ulogom u regulisanju aktivnosti VKOR. Oni su prikazali četiri bolesnika sa rezistencijom na varfarin sa različitim mutacijama ovog gena (Val29Leu, Val45Ala, Arg58Gly i Leu128Arg).

Od tada su se proširila saznanja o mutacijama gena za VKORC1 u interindividualnoj reaktivnosti i rezistenciji na terapiju varfarinom. Do sada je kod ljudi izdvojeno 10 najčešćih singl-nukleotid polimorfizama za VCORC1 koji se javljaju kod oko 5% ljudi. Istovremeno, izdvojeno je pet najčešćih halotipova (H1, H2, H7, H8, H9) od kojih su dva (H7 i H9 halo-

tipskih sekvencija TCGGTCCGCA i TACGTTCGCG) odgovorna za povećanje doze varfarina radi ostvarivanja očekivanog terapijskog odgovora [15]. Pored utvrđivanja mogućih mutacija danas se pažnja posvećuje utvrđivanju značaja, pojedinih genskih mutacija odgovornih za rezistenciju na varfarin prema učestalosti [16].

Kod prikazane bolesnice nije predstavljala problem samo rezistencija na oralne antikoagulantne lekove, već njeno neadekvatno zbrinjavanje. Bolesnici je savetovano da nastavi sa primenom niskomolekulskog heparina koji je ona samoinicijativno koristila u dozi dva puta nižoj od savetovane što je rezultovalo neokluzivnom trombozom valvule.

Zbog svojih farmakokoinetkih i farmakodinamskih svojstava, niskomolekulski heparin predstavljao je primamljivu alternativu kod bolesnika sa mehaničkim valvulama kojima je bilo potrebno prekinuti davanja oralnih antikoagulantnih sredstava. Međutim, klinička potvrda njegove sigurne i efikasne primene izostala je kod disk valvula plasiranih na mitralnoj poziciji i kod pridružene apsolutne aritmije, tako da se prednost daje primeni standardnog heparina [17].

Na osnovu iznetih podataka o problemu rezistencije na oralne antikoagulantne lekove može se zaključiti da kod propisivanja ovih lekova treba imati uvid u kliničko stanje i navike bolesnika. Jasno je da pridružene bolesti, način ishrane, unos alkohola, telesna masa i pridružena terapija utiču na ostvarivanje adekvatnog terapijskog odgovora. Nesumnjivu ulogu ima i genetski polimorfizam, kako u pojavi senzitivnosti, tako i u pojavi rezistencije na lek. Otkriće gena za VKOR rasvetlilo je u značajnoj meri genetsko poreklo rezistencije i interindividualne razlike u osetljivosti na primenjenu dozu leka. Međutim, navedeni napredak u saznanjima ne prati rutinsko ispitivanje genetskog porekla rezistencije na varfarin ni u razvijenijim delovima sveta.

Zaključak

Kada ne postoje pridružene komplikacije kao što je apsolutna aritmija, nalaz rezistencije na oralna atikoagulantna sredstva indikacija je za zamenu mehaničke proteze biološkom, što je i učinjeno kod prikazane bolesnice.

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LETTER TO THE EDITOR / PISMO UREDNIKU

Clinical experience: approach to a patient during physical examination

Iskustvo kliničara: odnos prema bolesniku tokom pregleda

People are the same, only a disease makes them differ in the normal enviroment and behaviour. Every patient and his/her disease is a labyrinth. The doctor only can find the way out, sometimes without any help but just relying on his own skills in examining the patient which actually represents an approach to a patient and acquiring valuable information within physical examination.

Being sick, every patient differs in behaviour and opinion about his illness when presenting to a doctor. Some are eager to get help, will ready answer questions, explain their difficulties, (sometimes in more details than necessary), some are affraid, some are quiet, some have the subconcious resistance to being sick. Most important, not all patients are on the same intelectual level. Also, bear in mind that some patients like a strict, calm and quite doctor, while others like a doctor who is professionally relaxed, makes a decent joke here and there just to relax the patient during the examination. The most important thing that a patient is looking in a doctor is confidence. Once the confidence is established in doctor/patient relationship, the every treatment becomes easier and more relaxed for the doctor and for the patient. So, the main point is that a doctor should be a pschycologist, able to "screen" a patient after a short conversation with him in order to evaluate his state of intelectual level and type of character. These are some practical advices based in everyday experiance with patients:

– Be kind, smile and be kind.

– for the reaction of the patient – whether it will make him smile, relax him or make him more tense which will give you good approximation of his character (if the reaction is positive it can have only good effect on relaxing a patient).

– Form your questions as simple as possible and understandable for a patient but useful for your evaluation of a patient´s disease.

– Do not frown when listening to a patient or examining him – it may scare him thinking that you discovered something wrong.

– Never use medical terms in conversation with a patient.

– Also, always insist that a patient also uses common terms when "explaining his difficulties (for example: if a patient says to have arrhythmia, comment by asking him if his heart beats regulary but fast or makes pauses in beating which will reveal simply whether patient has arrythmia or just tachycardia).

– If a patient is accompanied by someone, do not let the other person talk instead of a patient or at the same time, unless in cases when a patient is incapable of understandable speech. If a patient can speak for himself let him explain everything of interest to you guided by your "target" questions, and then you can fullfil the information by some observations an accompaning person might have.

– It is very useful for every doctor to know at least a few words of at least two or three foreign languages in case a patient does not speak your language.

– Never leave a patient in suspense about your plans of further treatment or diagnostics – in simple language explain your further plan with a patient and explain shortly but simply most important facts a patient should know about his disease and special diets or regimens he must commit to if necessery.

In case a patient was not speak any of the languages you speak, use improvised "hand language". For example, put yor finger or plam of the hand on the region of the patient´s body where you suspect he has difficulties or pains, shake your head or hand if you suspect of vertigo and so on – use all your immagination and improvise.

The other side, being part-pscyhologist, is that every doctor must be a "detective". You will always meet patients that for some reason (sometimes subconciesly, think that by not telling he will feel better) do not accept their illness, so they tend to deny problems that you ask about. In such cases use trick questions that will guide you to the right answer and real situation of the patient. For example if you ask the patient whether he has chest pains and he says no, but you

suspect he does, turn the questions to other problem, and then suprise him with the right but differently formulated question, actually concealing the point you want to get to (it is like a “go around game” sometimes).

The final point is, you can learn these little “tricks of the trade” from a more experienced doctor, learn them by “mistake” method, based on your personal experiance or simply you are born with that talent and feeling for adaptive communication. After all, not everything is allowed in “love and war” but most importantly in curing the patient.

At the end, let us remind ourselves that medicine is not only reading and studying but born talent, as well.

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

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Primeri oblika referenci:

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

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Examples of references:

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

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