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Svake godine, 7. aprila, na godišnjicu osnivanja Svetske zdravstvene organizacije, obeležava se Svetski dan zdravlja, svaki put posvećen nekoj drugoj temi od vitalnog značaja za zdravlje celokupnog svetskog stanovništva. Ovogodišnji Svetski dan zdravlja posvećen je održanju i unapređenju zdravlja u starijem životnom dobu. Aktivnosti koje će se pod sloganom „Ageing and health: Good health adds life to years" sprovoditi širom sveta tokom ove godine treba da skrenu pažnju javnosti da je starost briga svakog pojedinca, bez obzira na uzrast, pol, naciju, veru ili društveni status, i da svatko od nas treba da dâ svoj doprinos unapređenju zdravlja starijih osoba kako bi mogli da vode što potpuniji i kvalitetniji život na zadovoljstvo sebe samih, svoje porodice i društva u celini.

Every year World Health Day is celebrated on 7 April to mark the anniversary of the founding of World Health Organisation, each time dedicated to a theme of vital significance for the global population of the world. The topic of World Health Day this year is Ageing and health. Activities under the slogan "Ageing and health: good health adds life to years" should make the public pay attention to ageing concering each and every one of us regardless the age, gender, nationality, religion and social status, as well as to contribute to the improvement of older people health to let them lead full and productive lives to their own and the pleasure of their families and society in general.

ORIGINAL ARTICLE

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Uspeh intrauterusne inseminacije kod bolesnica sa nepoznatim uzrokom neplodnosti

Success rate of intrauterine insemination in patients with unknown infertility

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Apstrakt

Uvod/Cilj. Infertilitet nepoznatog porekla zastupljen je kod 10–26% infertilnih brakova. Prognoza za parove sa neobjašnjenim infertilitetom zavisi od mogućnosti da se eventualni neotkriveni defekat može korigovati tokom vremena. Intrauterusna inseminacija (IUI) i stimulacija ovulacije su metode izbora u tretmanu neobjašnjenog infertiliteta, a ukoliko je žena starija od 37 godina, može joj se direktno preporučiti *in vitro* fertilizacija (IVF). Cilj istraživanja bio je da se uporede rezultati ostvarenih trudnoća nakon IUI kod bolesnica sa neobjašnjenim uzrokom infertiliteta i sa blagim oblikom endometrioze. **Metode.** Ovom studijom obuhvaćeno je 50 bolesnica sa dijagnozom blagog oblika endometrioze (grupa A) i 50 sa neobjašnjenim infertilitetom (grupa B). Primenjena je stimulacija humanim menopauznim gonadotropinima (hMG) i indukcija horionskim gonadotropinima (hCG) prema istom terpijskom protokolu, i po istom metodu izvedena IUI. **Rezultati.** Procenat ostvarenih ovulacija bio je veći u grupi B ($p < 0,05$). U toku tri uzastopna ciklusa u grupi A ostvareno je ukupno osam (16%), a u grupi B devet (18%) trudnoća. U toku tri stimulisana uzastopna ciklusa izvedeno je ukupno 102 IUI u grupi A i 97 IUI u grupi B. U grupi A uspešno je izneto šest jednoplodnih i jedna blizanačka trudnoća (14%), dok je u grupi B uspešno završeno devet (18%) jednoplodnih trudnoća. **Zaključak.** Primena kombinacije kontrolisane ovarijalne hiperstimulacije i IUI predstavlja efikasni, jeftini i bezbedni način lečenja neplodnih parova, naročito kod parova sa neobjašnjenim infertilitetom. Blagi oblik endometrioze, kao etiološki faktor neplodnosti, negativno utiče na ishod postupka IUI.

Ključne reči:
neplodnost; endometrioza; inseminacija, veštačka; ovulacija, indukcija; prognoza.

Abstract

Background/Aim. Unknown cause of infertility exists in 10%–26% of couples with infertility problems. Treatment of these couples depends on the posibility of correcting the unidentified defect over time. Intrauterine insemination (IUI) and ovaluation stimulation are methods of choice in treatment of unexplained fertility, but if a woman is older than 37 years, *in vitro* fertilization (IVF) could be directly recommended. The aim of this research was to compare the success rate of pregnancies with IUI between the patients with unexplained infertility and the patients with mild form endometriosis. **Methods.** The study included on 50 patients diagnosed with mild form endometriosis (group A) and 50 patients with unknown cause infertility (group B). Using the same therapeutical protocol, human menopausal gonadothropin (hMG) stimulation and horionic gonadropin (hCG) induction were applied, as well as IUI. **Results.** The percentage of achieved ovulation was higher in the group B ($p < 0.05$). During the 3 simulated sequential periods 102 IUI were performed in the group A and 97 IUI in the group B. In the group A there were 6 single and 1 twin pregnancies sucesfully conceived (14%), while in group B there were 9 (18%) single pregnancies. **Conclusion.** The use of a combination of controled ovarian hyperstimulation and IUI is an effective, cheap and safe method for treating infertility couples, especially couples with unknown cause infertility. Mild form endometriosis, as etiological infertility factor, has a negative impact on IUI success rate.

Key words:
infertility; endometriosis; insemination, artificial; ovulation induction; prognosis.

Uvod

Infertilitet nepoznatog porekla zastupljen je kod 10–26% infertilnih brakova [1], a u 3–4% ovih slučajeva u pitanju je, ipak, imunološki faktor infertiliteta. Iz mnogih razloga prevalencija neobjašnjenog infertiliteta, navedena u literaturi veoma je različita [2].

Prognoza kod parova sa neobjašnjenim infertilitetom zavisi od mogućnosti da se eventualni neotkriveni defekat može korigovati tokom vremena. U nekim slučajevima reč je

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o teškom defektu, a mogućnost korekcije ravna je nuli. Kumulativni nivo trudnoće za parove sa neobjašnjenim infertilitetom za period 1–3 godine, računajući od njihove prve posete ginekologu iznosi 13–40% [2]. Šanse za trudnoću smanjuju se kod žena koje nikad nisu bile trudne (primarni infertilitet), ako su starije od 30 godina, ili ako infertilitet traje duže od tri godine [3].

Optimalna procena kod neplodnog para zavisi od protokolarne procene fertilnog potencijala oba partnera. Kompletno ispitivanje podrazumeva primenu velikog broja dijagnostičkih testova: istorija bolesti, fizikalni nalazi, ispitivanje seminalne tečnosti i funkcije spermatozoida, ispitivanje ovulatorne funkcije, integritet genitalnog trakta, detetkcija pratećih bolesti (infekcije), kao i dodatna ispitivanja u cilju otkrivanja uzroka azospermije, amenoreje, hiperprolaktinemije, itd [2].

Svaki tretman je uspešan i zadovoljavajući ukoliko je racionalan, a terapija je efikasna ukoliko koriguje defekte i dovede do željenog ishoda. Da li će se jednom paru predložiti medicinski tretman ili čekanje spontane trudnoće, zavisi od dužine infertiliteta, starosti žene i želje para. Žene starije od 35 godina trebalo bi da počnu medicinski tretman u cilju ostvarivanja trudnoće pre nego mlađe žene [3].

Intrauterusna inseminacija (IUI) i stimulacija ovulacije predstavljaju metode izbora u lečenju neobjašnjenog infertiliteta, a ukoliko je žena starija od 37 godina, može joj se odmah preporučiti *in vitro* fertilizacija (IVF) [2, 3]. Intrauterinska inseminacija je manje invazivna i jeftinija nego IVF i njome se može postići trudnoća kod 10–17% slučajeva prema ciklusu, sa 85% trudnoća tokom prva četiri ciklusa [4].

Metoda IUI zajedno sa ovarijalnom stimulacijom predstavlja jednostavno i ne tako skupo lečenje infertiliteta. Zvanično objavljeni radovi ukazuju da stopa trudnoće prema ciklusu varira između 8 i 22%. Velika razlika u stopi trudnoće kod različitih autora može biti posledica male populacije ispitanika, razlike u karakteristikama ispitanika, različitim protokolima ovarijalne stimulacije i različitih tehnika inseminacije [4].

Humani menopauzni gonadotropin (hMG) primenjuje se u cilju stimulacije kod poremećene funkcije hipotalamusa, hipofize, odnosno kod bolesnica sa anovulacijom kod kojih prolaktin nije povišen, sa hipogonadotropnom amenorejom, sa manjkom gonadotropina u urinu, niskim estrogenima, negativnim progesteronskim testom i kod onih kod kojih je oralna terapija klomifenom bila bezuspešna [5].

Cilj našeg istraživanja bio je da uporedimo rezultate ostvarenih trudnoća nakon kontrolisane hiperstimulacije ovarijuma hMG i IUI kod bolesnica sa neobjašnjenim uzrokom infertiliteta i kod bolesnica sa blagim oblikom endometrioze.

Metode

Ova prospektivna studija sprovedena je od 01. 12. 2004. do 01. 12. 2006. u Ginekološko-akušerskoj klinici (GAK) „Narodni front“, Beograd i obuhvatila je 100 infertilnih bolesnica prosečnih godina 32,57 ± 4,6 (22–40 godina). Bolesnice su bile podeljene u dve grupe: grupu A od 50 bolesnica sa blagim oblikom endometrioze (prethodno laparoskopski dijagnostikovan) i grupu B od 50 bolesnica sa neobjašenjenim uzrokom infertiliteta.

Istraživanje je bilo uslovljeno sledećim faktorima: starost bolesnica ≤ 40 godina, negativni test na antispermatozoidna antitela (ASA) u serumu oba supružnika, normalna prolaznost genitalnog trakta potvrđena prethodnom celioskopijom i histerosalpingografijom, normalni hormonski profil u folikularnoj fazi ciklusa, regularni menstrualni ciklusi (dužina ciklusa između 25 i 35 dana), infertilitet koji traje > 12 meseci. Sve bolesnice bile su zdrave, bez hroničnih bolesti, bez operativnih zahvata na tubama, uterusu i sl.

Bolesnice sa minimalnim ili blagim oblikom endometrioze bile su uključene u studiju šest meseci nakon tretmana.

Za supružnike bilo je neophodno prisustvo odgovarajućeg broja pokretnih spermatozoida (akcenat na broju, morfologiji i pokretljivosti spermatozoida). Da li je kvalitet sperme bio u granicama normale ili ispod nje određivano je prema modifikovanom vodiču Svetske zdravstvene organizacije SZO (1987, 1992, 1999), a definisan je normalni nalaz na sledeći način: broj spermatozoida: > 20 000 000/mL, normalne forme > 30%, i progresivna pokretljivost > 40%. Ovi parametri odnosili su se na kvalitet semena pre obrade sperme za IUI. Ukoliko je broj progresivno pokretnih spermatozoida nakon obrade sperme bio manji od 1 000 000, ti su parovi isključivani iz studije [6]. Svi supružnici bili su zdravi, bez hroničnih bolesti, bez operativnih zahvata na testisima i sl.

Kod svih infertilnih parova prvi put je u cilju terapije infertiliteta sprovedena hiperstimulacija gonadotropinima (hMG) i IUI.

U obe ispitivane grupe primenjeni su uniformni protokoli stimulacije hMG-om, indentični u odnosu na vreme započinjanja, vrstu i dozu preparata i prilagođeni svakoj bolesnici.

Od preparata hMG upotrebljen je Menopur® (Ferring Pharmaceutical) koji sadrži 75 IJ folikulostimulišućeg hormona (FSH) i 75 IJ lutenizirajućeg hormona (LH). U cilju indukcije ovulacije kao humani horionski gonadotropin upotrebljen je preparat Pregnyl® (Organon).

Stimulacija hMG rađena je od 2. do 7. dana ciklusa davanjem prva dva dana po tri, a zatim po dve ampule hMG do zadovoljenja ultrasonografskih kriterijuma za davanje hCG u dozi od 5 000 IJ. Uslov za administraciju hCG bio je serumski nivo E2 ≤ 3000 pg/mL.

Posle IUI bolesnice nisu imale suplementaciju lutealne faze.

Ovarijalni i endometrijalni odgovor na hiperstimulaciju hMG praćen je transvaginalnom ultrasonografijom (TV-US) koja je obavljena u kabinetu za TV-US GAK ”Narodni front” u Beogradu. Upotrebljen je ultrazvučni aparat Hitachi sa transvaginalnom sondom jačine 5,5 MHz, a preglede su obavila tri lekara na istom ultrasonografskom aparatu.

Kod svih bolesnica pre započinjanja stimulacije ovulacije rađena je bazalna folikulometrija neposredno po završetku menstruacije (3–5 dan). Praćenje rasta i razvoja folikula i debljine endometrijuma vršeno je od 8. dana ciklusa.

Ultrasonografski kriterijumi za administraciju hCG bili su: najmanje jedan folikul promera ≥ 18 mm (ali ne vi-

še od 8 folikula) i debljina endometrijuma ≥ 6 mm. Ultrasonografski ovulaciju smo konstatovali kao kolabiranje dominantnog folikula i prisustvo slobodne tečnosti u retrouterinom prostoru.

Sperma za IUI dobijena je masturbacijom i skupljena u sterilnoj posudi nakon apstinencije od 2 do 4 dana, 1h pre pripreme za IUI. Nakon likvefakcije na sobnoj temperaturi i analize kvaliteta semena (broj, morfologija i pokretljivost spermatozoida), pažljivo je sipano 1 mL semena i 2 mL medijuma (Menezo B2) u sterilnu epruvetu. Zatim, centrifugiranjem (broj obtraja od 250 x g u trajanju od 10 minuta) vršilo se, praktično ispiranje pokretnih, vitalnih spermatozoida, koji su činili supernatant, dok su nepokretni spermatozoidi i ćelijski detritus ostali na dnu epruvete.

U dobijeni i izdvojeni supernatant dodato je 2,5 mL medijuma, nakon čega se vršilo recentrifugiranje u trajanu od 10 minuta. Dobijenom supernatantu pažljivo se dodalo 1 mL medijuma i epruveta stavila u termostat na temperaturu od 37°C 1–2 h. Tokom inkubacije optimizirani spermatozoidi zauzimali su gornji deo smeše, pa se uzorak za analizu i inseminaciju uzimao iz ovog dela.

Obično se IUI radi 36 h posle davanja hCG pomoću mini intrauteruskog katetera koji se nastavlja na sterilni plastični špric za jednokratnu upotrebu, zapremine 2 mL. Zapremina isprane sperme iznosi 0,5 do 2 mL.

Priprema i postavljanje svih bolesnica obavljena je na uobičajeni i isti način, u ginekološkom položaju. Nakon prikazivanja *portio vaginalis uteri* spekulumom, kateter se vrlo pažljivo i nežno preko cervikalnog kanala unosio u kavum uterusa, nakon čega se lagano ubrizgala obrađena sperma. Bolesnice su bile postavljene u lagani Trendelerburgov položaj i ostajale tako 20–30 minuta.

U slučaju izostanka menstruacije nakon IUI, određivan je nivo β hCG u serumu 14 dana nakon IUI. Pozitivnim nalazom smatrali smo vrednosti ≥ 10 mIU/mL. u periodu od 14. do 16. dana. Sve trudnoće smo ultrasonografski potvrdili, sa prisutnom srčanom radnjom.

Za statističku obradu rezultata, pored deskriptivne metode, korišćeni su statistički parametarski i neparametarski testovi: Studentov *t*-test, χ^2 test i metoda jednofaktorske neparametarske analize varijanse za proporcije. Nivo statističke značajnosti bio je $p = 0,05$.

Rezultati

U tabeli 1 date su demografske karakteristike ispitanica.

Ispitano je ukupno 100 bračnih parova. Metodom jednofaktorske neparametarske analize varijanse za proporcije, testirali smo značajnost razlike učestalosti kategorija različitih životnih doba kod ispitanica i njihovih supružnika. Dobijene vrednosti (F = 3,458 za ispitanice i F = 2,112 za njihove supružnike), govore da razlika u učestalosti prema godinama starosti nije bila statistički značajna ($p > 0.05$), kao ni razlika unutar svake grupe ponaosob. Grupe su bile homogene, međusobno slične i *a priori* poredive.

Tabela 1

Karakteristike ispitivanih grupa

Karakteristike ispitanica	Grupa A (n = 50)	Grupa B (n = 50)	*p*
Starost ispitanica (godine)*	32 ± 4,47 (24–38)	33,14 ± 4,47 (22–38)	
21–25**	10	4	
26–30	24	30	> 0,05†
31–35	42	32	
≥ 36	24	34	
Starost supružnika (godine)*	35,98 ± 4,74 (28–46)	37,8 ± 5,08 (26–47)	
26–30	16	8	
31–35	32	32	
36–40	30	24	> 0,05†
41–45	20	30	
≥ 46	2	6	
Dužina trajanja infertiliteta (meseci)*	37,3 ± 14,27 (16–70)	34,6 ± 13,05 (15–70)	
12–24**	26	28	> 0,05†
25–36	16	30	< 0,05‡
≥ 37	58	42	

Grupa A – blagi oblik endometrioze; Grupa B – neobjašnjeni uzrok infertiliteta
*** vrednosti su prikazane kao srednja vrednost, standardna devijacija i raspon;**
**** frekvencije izražene u procentima (%);**
† primenjena je jednofaktorska neparametarska analiza varijanse za proporcije;
‡ unutargrupna razlika distribucije grupe A testirana je χ^2 testom

Ovo istraživanje uključilo je bračne parove u kojima su supružnici imali normospermiju, odnosno svi su imali broj spermatozoida preko 20 000 000/mL, normalne forme > 30%, i progresivnu pokretljivost > 30%. Karakteristike spermograma prikazane su u tabeli 2.

Prosečan broj spermatozoida u mL kod supružnika grupe A bio je 62,5 ± 34,106 (medijana 55; raspon 20–135 miliona/mL). Prosečan broj spermatozoida/mL kod supružnika grupe B bio je 72,02 ± 38,091 (medijana 80; raspon 20–140 miliona/mL).

Metodom jednofaktorske neparametarske analize varijanse za proporcije testirali smo značajnost razlike učestalosti kategorija različite količine spermatozoida u mL kod ispitanika grupe A i B. Dobijena je vrednost F = 3,202 ($p > 0,05$). Razlika u učestalosti po kategorijama broja spermato-

Tabela 2

Kvalitet semena ispitanih supružnika

Ispitani parametri	Grupa A (n = 50)	Grupa B (n = 50)	*p*
Broj spermatozoida/mL*	62,5 ± 34,106	72,02 ± 38,091	
20 000 000–40 000 000**	30	30	> 0,05†
40 000 001–60 000 000	32	14	< 0,05
> 60 000 000	38	56	
Progresivna pokretljivost spermatozoida (%)*	61,6 ± 9,92	55,36 ± 16,925	
30–40**	2	28	
41–50	20	20	< 0,05†
≥ 51	78	52	
Normalna morfologija (%)*	53 ± 10,101	50,98 ± 16,821	
30–40	16	40	> 0,05†
41–50	38	12	< 0,05
≥ 51	46	48	

Grupa A – blagi oblik endometrioze; Grupa B – neobjašnjeni uzrok infertiliteta
***vrednosti su prikazane kao srednja vrednost, standardna devijacija i rang;**
**** frekvencije izražene u procentima (%);**
† primenjena metoda jednofaktorske neparametarske analize varijanse za proporcije;
‡ unutargrupna razlika testirana metodom χ^2 testa

zoida nije statistički značajna. Daljom analizom testirali smo razliku distribucije unutar grupe A, metodom χ^2 testa slaganja, i ta razlika bila je značajna ($p < 0,05$) za kategoriju od > 60 000 000/mL.

U tabeli 3 prikazani su rezultati stimulacije pomoću hMG u obe grupe, broj odigranih ovulacija prema ciklusu, kao i broj ostvarenih trudnoća.

Tabela 3

Stimulacije, ostvarene ovulacije i trudnoće

Grupe bolesnica	Broj stimulacija (n)	Broj bolesnica po ciklusu (n)	Ostvarene ovulacije (%)	Ostvarene trudnoće (%)
Grupa A (n = 50)	1	50	74	8,11
	2	45	77,78	5,71
	3	40	75	10
Grupa B (n = 50)	1	50	82	9,76
	2	38	84,21	9,38
	3	28	85,71	8,33

Grupa A – blagi oblik endometrioze; Grupa B – neobjašnjeni uzrok infertiliteta

U grupi A prosečna vrednost progresivno pokretnih spermatozoida bila je 9,92 ± 61,6% (min. 40%, max 80%; medijana = 60%), a u grupi B 16,925 ± 55,36% (min. 30%, max 80%; medijana = 57.5%).

Metodom jednofaktorske neparametarske analize varijanse za proporcije testirali smo značajnost razlike učestalosti kategorija različite pokretljivosti spermatozoida kod ispitanika grupe A i B. Razlika u učestalosti po kategorijama pokretljivosti spermatozoida bila je statistički značajna (F = 7,231; $p < 0,05$). Daljom analizom testirali smo razliku distribucije unutar grupa i utvrđeno je da su bolesnici grupe A imali u proseku pokretljivije spermatozoide.

Našli smo da je u grupi A prosečna vrednost spermatozoida sa normalnom morfologijom iznosila 53 ± 10,101% (35–80%; medijana 50%), a u grupi B 50,98 ± 16,821% (30–80%; medijana 50%).

Metodom jednofaktorske neparametarske analize varijanse za proporcije utvrdili smo da razlika u učestalosti po kategorijama pokretljivosti spermatozoida između grupa nije bila statistički značajna (F = 2,482; $p > 0.05$). Metodom χ^2 testa slaganja testirali smo razliku distribucije unutar grupa A i B, i ta razlika bila je značajna ($p < 0.05$). U obe grupe preovladavali su spermatozoidi normalne pokretljivosti.

Metodom jednofaktorske neparametarske analize varijanse za proporcije testirali smo značajnost razlike učestalosti kategorija ovulacija po cilkusu kod bolesnica grupe A i B. Dobijena je vrednost F = 8,322, što znači da je razlika u učestalosti ovulacije bila statistički značajna, tj. veći je procenat ostvarenih ovulacija bio u grupi B. Unutar svake grupe, ponaosob, testirana je značajnost razlike u procentu ostvarene trudnoće. Grupa B bila je homogena, a grupa A heterogena u odnosu na broj stimulacija (najmanji procenat ostvarenih trudnoća zabeležen je kod žena koje su imale po 2 stimulacije).

Dobijeni rezultati pokazali su da je u toku tri uzastopna ciklusa u grupi A ostvareno ukupno osam trudnoća (16%), a u grupi B devet (18%).

U toku tri stimulisana uzastopna ciklusa izvedeno je ukupno 102 IUI u grupi A i 97 u grupi B. Ishod postupka IUI prikazan je u tabeli 4.

Razlika u učestalosti po kategorijama ishoda IUI između grupa nije bila statistički značajna, već samo razlika unutar svake grupe ponaosob ($p < 0,05$).

Ako uzmemo kao dobar ishod trudnoće rađanje živog deteta, u grupi A uspešno je izneto šest jednoplodnih i jedna blizanačka trudnoća (14%), dok je u grupi B uspešno završeno devet (18%) jednoplodnih trudnoća ($p > 0.05$).

Učestalost trudnoće u zavisnosti od karakteristika trudnica i karakteristika spermograma, prikazana je u tabeli 5.

Tabela 4

Ishod intrauterusnih inseminacija (IUI)

Ishod IUI	Grupa A (n = 50)	Grupa B (n = 50)
	%	%
Bez trudnoće	84	82
Ab. spontaneus	2	0
Jednoplodna trudnoća sa živorođenim detetom	12	18
Višeplodna trudnoća sa živorođenim detetom	2	0

Grupa A – blagi oblik endometrioze; Grupa B – neobjašnjeni uzrok infertiliteta

Tabela 5

Stopa trudnoće nakon intrauterusne inseminacije

Parametri	Ukupan broj bolesnica (n)	Broj ostvarenih trudnoća (n)	Stopa trudnoće (%)
Uzrast ispitanica (god)			
≤ 35	71	15	21,13
≥ 36	29	2	6,9
Dužina trajanja infertiliteta (meseci)			
12–24	27	9	33,34
25–36	23	6	26,10
> 37	50	2	4
Uzrok infertiliteta			
endometrioza	50	8	16
neobjašnjeni infertilitet	50	9	18
Broj spermatozoida (milion/mL)			
20–40	30	2	6,67
40–60	23	5	21,74
> 60	47	10	21,28
Progresivna pokretljivost spermatozoida (%)			
30–40	15	2	13,34
40,1–50	20	5	25
> 50	65	10	15,38

Diskusija

Većina neplodnih bračnih parova praktično su supfertilni, sa smanjenom mesečnom stopom trudnoće, ali sa istovremenom mogućnošću spontane trudnoće. U planiranju lečenja infertiliteta treba uvek svesti na minimum moguće zdravstvene rizike, koji su najčešće udruženi sa ovarijalnom stimulacijom, ali istovremeno uzeti u obzir i cenu koštanja celokupnog lečenja. Kao primer, *in vitro* fertilizacija (IVF) i intracitoplazmatska spermalna injekcija (ICSI) bili su inicijalno korišćeni u slučajevima bilateralne tubarne opstrukcije kod teških tubarnih oštećenja ili teških oblika muškog infertiliteta. Želja da se što pre dođe do uspeha, praktično je proširila indikacije ovih metoda. Međutim, to nije značilo da je kod tih proširenih indikacija primena ovih invazivnih metoda obećavala veći uspeh.

Zajedno sa ovarijalnom stimulacijom IUI predstavlja jednostavnu i jeftinu metodu lečenja infertiliteta nepoznate etiologije kod blagog oblika endometrioze, kao i u prisustvu muškog faktora infertiliteta, ali sa prisustvom dovoljnog broja progresivno pokretnih spermatozoida [4, 7].

U toku ovog istraživanja praćene su dve grupe ispitanica: sa minimalnim oblikom endometrioze (grupa A) i sa nepoznatim uzrokom neplodnosti (grupa B).

Obe grupe ispitanica stimulisane su sa hMG, uz primenu uniformnih protokola. Adekvatnim tokom stimulacije smatran je onaj u kome je u najmanje dve folikule bio započet rast i ostvaren kontinuiran porast veličine folikula do postizanja kriterijuma za primenu humanog horionskog gonadotropina.

Životna dob ispitanica veoma je važan prognostički faktor kod primene postupka IUI. Naši rezultati pokazuju da je najveći broj trudnoća ostvareno u dobi ≤ 35 godina. Naime u obe grupe ispitanica, ostvareno je ukupno 17 trudnoća. Od toga je čak 15 (88,24%) trudnoća ostvareno kod ispitanica mlađih od ≤ 35 godina, a samo 2 (11,76%) trudnoće u dobi ≥ 36 godina. Ovi rezultati ukazuju na to da je nakon 36. godine života najverovatnije smanjena receptivnost uterusa i/ili smanjen kvalitet oocita. Naši podaci slažu se sa rezultatima drugih autora, dok neki autori pomeraju granicu na 40 godina i smatraju da stopa trudnoće opada nakon 40 godine. U svakom slučaju, ovi autori napominju da primena IUI, kao načina lečenja infertiliteta nakon 40. godine daje loše rezultate [7–10].

Dužina infertiliteta značajan je prognostički faktor u tretmanu infertiliteta.

U našem ispitivanju od 27 bračnih parova, kod kojih je infertilitet trajao 12–24 meseca ostvareno je ukupno osam trudnoća. Kod 23 para infertilitet je trajao 25–36 meseci (ostvareno je sedam trudnoća) a kod 50 parova je infertilitet trajao ≥ 37 meseci (ostvarene su dve trudnoće 4%). Drugim rečima, lečenje infertiliteta kod naših ispitanika bilo je naju-

spešnije ako je njegova dužina bila manja od 37 meseci. Naši rezultati poklapaju se sa rezultatima drugih autora [4, 7].

U naše istraživanje bili su uključeni bračni parovi u kojima su supružnici imali normospermiju, uz pridržavanje striknih kriterijuma SZO [6]. Naši podaci govore da što je veći broj spermatozoida i što je veći njihov motilitet, to je uspeh lečenja bolji. Najveći procenat ostvarenih trudnoća bio je u kategoriji spermatozoida preko 40 000 000/mL i preko 40% pokretljivosti. Naši rezultati poklapaju se sa brojnim radovima različitih autora [6, 7, 9]. Neki autori napominju da je stimulacija ovulacije u kombinaciji sa IUI metoda izbora kod brojnih uzroka ženskog i muškog infertiliteta, ali da je neophodno da broj pokretnih spermatozoida obrađenih za inseminaciju bude preko 1 000 000/mL [10, 11].

U grupi ispitanica sa blagim oblikom endometrioze bilo je 50 kod kojih su ciklusi praćeni ultrasonografski. Tokom prve stimulacije ovulacija se odigrala kod 37 (74%) ispitanica. Nakon inseminacije kod 37 ostavrene su tri (8,11%) trudnoće. Dva puta stimulisano je 45 žena, a ovulacija se odigrala kod 35 (77,78%). Nakon inseminacije kod žena sa odigranom ovulacijom ostvarene su dve (5,71%) trudnoće. Tri puta stimulisano je 40 žena, a ovulacija se odigrala kod 30 (75%), pri čemu kod njih nakon IUI ostvarene su tri trudnoće odnosno 10%.

U grupi žena sa nepoznatim uzrokom infertiliteta bilo je takođe 50 žena. Ovulacija se odigrala kod 41 (82%). Kod žena sa odigranom ovulacijom nakon IUI ostvareno je četiri trudnoće (9,76%). U grupi B dva puta je stimulisano 38 žena, a ovulacija se odigrala kod 32 (84,21%). Nakon inseminacije kod žena sa odigranom ovulacijom, ostvareno je tri (9,38%) trudnoće. Tri puta u grupi B stimulisano je 28 žena, a ovulacija se odigrala kod 24 (85,71%), pri čemu su nakon IUI ostvarene dve (8,33%) trudnoće.

Razlika u učestalosti po kategorijama procenata ovulacije nije statistički značajna, kao ni razlika unutar svake grupe ponaosob, iako nešto veći procenat ovulacije odigrao se u grupi B. Grupe su bile homogene, međusobno slične i *a priori* poredive u odnosu na ovulaciju.

Hiperstimulaciju hMG-om, kao efikasnu metodu za realizaciju visokog procenta ovulacije, nalazimo i u radovima drugih autora [5, 8, 12–14].

U toku tri stimulisana uzastopna ciklusa izvedeno je ukupno 102 IUI u grupi A i ostvareno ukupno osam (16%) trudnoća, odnosno 7 (14%) trudnoća sa živorođenom decom. U grupi B urađeno je ukupno 97 IUI u toku tri uzastopna ciklusa, i u toku istih ostvareno ukupno devet (18%) trudnoća, odnosno sve su bile uspešne i rezultovale živorođenom decom. Razlika u učestalosti po kategorijama ishoda IUI nije bila statistički značajna, već samo razlika unutar svake grupe ponaosob ($p < 0,05$).

Ako uzmemo kao dobar ishod trudnoće živorođeno dete, u grupi A ta učestalost bila je 14%, a u grupi B 18% ($p > 0,05$).

Efekat etiologije neplodnosti veoma je značajan faktor. Naši rezultati bili su bolji kod nepoznate etiologije infertiliteta, u odnosu na endometriozu. Negativni uticaj endometrioze, makar i minimalnog i blagog oblika, navode brojni autori. Neki autori zaključuju da blagi i minimalni oblik endometrioze smanjuju efekat tretmana ovarijalne kontrolisane hiperstimulacije/IUI za približno 50%, u odnosu na parove kod kojih je uzrok infertiliteta neobjašnjene prirode ili muški faktor, što je u skladu i sa našim rezultatima [7, 9].

Primenu kombinacije kontrolisane ovarijalne hiperstimulacije i IUI kao efikasni način lečenja kod neplodnih parova svojim radovima potvrđuju i drugi autori [14, 15].

Analizirajući ishoda IUI između grupa, našli smo da je stopa trudnoće veća u grupi trudnica sa nepoznatim uzrokom infertiliteta (18%) u odnosu na grupu sa blagim oblikom endometrioze (16%). Visoki kvalitet semena kod naših ispitanika obećava bolji uspeh nakon IUI. Upoređivanjem podataka broja ostvarenih trudnoća u odnosu na karakteristike žena i spermalne parametre dolazi se do zaključka da je stopa trudnoće kod žena ≤ 35 godina statistički značajno veća u odnosu na one preko 35 godina.

Zaključak

Primena kombinacije kontrolisane ovarijalne hiperstimulacije i IUI je efikasni, jeftini i bezbedni način lečenja neplodnih parova, naročito kod parova sa neobjašnjenim infertilitetom. Blagi oblik endometrioze, kao etiološki faktor neplodnosti, ima negativni uticaj na ishod postupka IUI.

Uspeh kombinacije kontrolisane ovarijalne hiperstimulacije/IUI, takođe, zavisi od starosti žena, kvaliteta semena i dužine trajanja infertiliteta.

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Factors influencing the choice of antidepressants: a study of antidepressant prescribing practice at University Psychiatric Clinic in Belgrade

Faktori koji utiču na izbor antidepresiva: analiza propisivanja na Univerzitetskoj psihijatrijskoj klinici u Beogradu

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Abstract

Background/Aim. Antidepressants are a widely used class of drugs. The aim of this study was to investigate different aspects of antidepressant prescribing practice at University Psychiatric Clinic in Belgrade. **Methods.** This cross-sectional study was carried out by retrospective analysis of the patient's medical charts. The study included all patients with antidepressant prescribed at discharge during 2009 (n = 296). The evaluation was focused on patient-related factors (socio-demographic and illness related), psychiatrist-related factors (sex and duration of working experience) and drug related factors (type of antidepressant, dose, polypharmacy and reimbursement by national health insurance). **Results.** Antidepressants were prescribed for unipolar depression (F32-34, ICD X) either without comorbidity (46.2%) or with comorbidity (24.7%), mostly as a monotherapy (91% had one antidepressant), to the patients who were 65% female, aged 50.1 ± 8.9, most of them with 12 years of education (52.6%), married (69.3%) and employed (55.9%). The majority of patients had a history of two hospitalizations (Med 2; 25th–75th perc. 1–4) during nine years (Med 9; 25th–75th perc. 2–15) after the first episode of depression. Among them, 19% were found to be suicidal in a lifetime. The single most prescribed antidepressant was sertraline (20.4%), followed by fluoxetine (13.3%) and maprotiline (11.7%). Utilization of antidepressants was positively correlated with the rate of reimbursement ($p < 0.01$). The most prescribed antidepressant group was selective serotonin reuptake inhibitors (SSRI) (47.8%), followed by tricyclic antidepresants (TCA) (25.3%) and new antidepressants – venlafaxine, tianeptine, mirtazapine, bupropion, trazodone (15.1%). Most of the drugs were prescribed in doses which are at the lower end of the recommended dose-range. Regarding severity of the actual depressive episode, TCA were prescribed for severe depression with psychotic features, while SSRI were choice for episodes with moderate symptom severity ($p = 0.01$). Psychiatrists with longer working age (20–30 years) hesitated to prescribe new antidepressants in comparison to younger colleagues ($p = 0.01$). **Conclusion.** Economic issues in Serbia as developing country influence the choice of antidepressants, as well as a psychiatrist's working age and severity of depression. However, SSRI are the drugs of the first choice, as it was shown in most of the developed countries nowadays.

Key words:
antidepressive agents; drug prescriptions; patients; physicians; pharmaceutical preparations.

Apstrakt

Uvod/Cilj. Antidepresivi (AD) su vrsta lekova koji se široko koriste u lečenju. Cilj rada bio je analiza propisivanja AD i faktora koji utiču na izbor leka. **Metode.** Retrospektivnom studijom obuhvaćeni su svi bolesnici sa propisanim AD na otpustu sa hospitalnog lečenja u Univerzitetskoj klinici u Beogradu tokom 2009. godine (n = 296). Analizirani su faktori koji se tiču bolesnika (sociodemografski podaci, istorija bolesti), psihijatra (pol i godine staža) i samog leka (vrsta, doziranje, polifarmacija, pozicija na listi lekova koji se izdaju na teret zdravstvenog osiguranja). **Rezultati.** Antidepresivi su bili najčešće propisivani za lečenje unipolarne depresije (70,9%), bez komorbiditeta (kod 46,2% bolesnika) ili sa komorbiditetom (2,7%), uglavnom kao monoterapija (≥ 2 AD kod 9%), bolesnicima koji su u 65% slučajeva bili ženskog pola, starosti 50,1 ± 8,9 godina, u proseku sa 12 godina obrazovanja (52,6%), u braku (69,3%) i u statusu zaposlenih (55,9%). Većina bolesnika bila je dva puta hospitalizovana (Med 2; Q1-Q3 1–4) u periodu od devet godina (Med 9; Q1-Q3 2–15) nakon prve epizode depresije. Ukupno 19% bolesnika bilo je suicidno do tada. Najpropisivaniji AD bili su sertralin

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(20,4%), fluoksetin (13,3%) i maprotilin (11,7%). Učestalost propisivanja korelisala je pozitivno sa pozicijom na listi lekova koji se izdaju na teret osiguranja ($p < 0.01$). Selektivni inhibitori preuzimanja serotonina (SSRI) bili su prepisani kod 47,8% bolesnika, triciklični antidepresivi (TCA) kod 25,3%, a „novi" AD (venlafaksin, tianeptin, mirtazapin, bupropion, trazodon) kod 15,1% bolesnika. Većina lekova propisivana je u najnižoj preporučenoj dozi. Kod psihotične depresije TCA su bili značajno češće prepisivani, dok su SSRI bili lekovi izbora kod umereno teške simptomatologije ($p = 0.01$). Psihijatri sa dužim radnim stažom (20–30 godina) ređe su propisivali "nove" AD ($p = 0.01$). **Zaključak.** Ekonomski faktori, dužina radnog staža psihijatra i težina depresivne epizode su faktori koji su uticali na izbor AD kod ispitivanih bolesnika. Podatak da se SSRI koriste najviše u skladu je sa praksom većine razvijenih zemalja.

Ključne reči:
antidepresivi; lekovi, propisivanje; bolesnici; lekari; lekovi.

Introduction

The use of antidepressants (ADs) has been steadily increasing during the last decade [1, 2]. Drugs from this group act predominantly on serotonergic and/or noradrenergic transmission and reduce a spectrum of symptoms – from affective and will-instinctive to cognitive psychopathology.

Following history of development of ADs, it is evident that tricyclic ADs (TCA) such as amitriptiline, clomipramine, maprotiline were the most significant drugs in the treatment of depression from the early sixties to the late nineties all over the world. Later on, the emphasis was on synthesis and the application of selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, sertraline, paroxetine, citalopram and escitalopram, whose indications are wider [3].

In the last fifteen years the group of ADs has been rapidly enlarged, so that in contrast to the former relatively small number of available drugs, today we are disposed to a large number of ADs. New ADs include a few groups of drugs: selective antagonist reuptake inhibitors (SARIs) (trazodone), noradrenaline dopamine reuptake inhibitors (NDRIs) (bupropion), serotonin noradrenaline reuptake inhibitors (SNRIs) (venlafaxine), noradrenergic and specific serotonergic antidepressants (NaSSAs) (mirtazapine).

Each new generation and class of ADs was expected to have better tolerability, greater safety and preserved efficacy. SSRIs proved to be minimally cardiotoxic in comparison to TCA, more suitable for geriatric population and with favourable dosage profile (autointoxication with TCA frequently appeared in the treatment of depression [4], while the latest ADs were characterised with better initial tolerability, in relation to SSRIs, as well as reduced frequency of side effects). New ADs have been accepted as a chance for theraporesistent cases, as well as for cases where given AD proved to be inefficient.

Enlarged group of ADs is undoubtedly an advantage for clinicians, but with the widen range the choice of the medicine is somehow complicated. Algorithms are an example of the attempt to include a certain coordination in the process of decision making, but it is worth mentioning that in the recently published study by Divac et al. [5] it was shown that only 41.5% of doctors at the University Psychiatric Clinics in Belgrade follow the therapeutic guidelines, published in 2004 [6]. The introduction of the therapeutic guidelines in Serbia is in progress. The legal aspects of its implementation will be carefully considered and regulated.

Even in the countries in which therapeutic guidelines were in use for several decades, there were a number of factors independent of the algorithms themselves to play a role in the choice of ADs[7]. The study [8] published in USA showed that the choice of drug was associated mostly with the type of clinical symptoms and with its side effects profile, while the price and the influence of a visiting representative of a drug manufacturer influenced the drug choice in lesser amount [8.] . On the other hand, psychiatrists from Asia considered demographic factors (patient's age, hospital addmission type) as more important for drug choice than the clinical symptoms [9]. Finally, the choice of AD in Europe [10] was associated with doctor's characteristics (age, specialization), as well as with the factors related to the patient (severity of depression, age, education, the existence of comorbidity).

By searching the literature, we noticed that there were no studies on AD prescription practice in Serbia so far, except a publication from Vojvodina written by Lisulov and Nedic [11] that analyzed practice of general practitioners (primary health care). Therefore, the aim of this study was to analyze prescribing of ADs at the University Psychiatric Clinic in Belgrade and to evaluate which ADs were most frequently used in relation to three types of factors: patient-related factors (sociodemographic and illness related), psychiatrist-related factors (sex and duration of working experience), and drug related factors (type of AD, dose, polypharmacy and reimbursement by health insurance).

Methods

This pharmacoepidemiological cross-sectional study was carried out by retrospective analysis of the patient's medical charts. Analyses included medical documentation of all subjects treated at the Clinic for Psychiatry, Clinical Centre of Serbia, Belgrade (in-patients and day-hospital patients) from January to September 2009 with ADs prescribed at discharge, regardless of the diagnosis.

Following medical chart data were considered: ADs and all other prescribed drugs, ADs daily dose, diagnosis, sex, age, marital status (married, single, divorced, widower), education (less than 12 years of education, more than 12 years of education), employment (employed, unemployed, pensioner, student), duration of the disease, number of hospitalizations and suicidal attempts in patient's history. In parallel, we noted the sex and working experience of psychiatrist who prescribed ADs (a total of 24 doctors; six had less than 10 years of serv-

ice, 11 had 10–19 years of service and 7 had more than 20 years of service). Finally, we considered economic factor by analyzing percentage of every AD refunded by health-insurance (The List of Drugs Prescribed and Issued at the Expense of the Obligatory Health Service – "positive list"; the source was "Official Gazzete of the Republic of Serbia", published in October 2009).

After all charts review, number of patients with at least one AD prescribed at discharge (from January to September 2009) was 296.

For some further analyses, ADs were grouped in the following way: TCAs (amitriptiline, clomipramine, maprotiline); SSRIs (fluoxetine, paroxetine, sertraline and escitalopram); new and the other ADs (venlafaxine, trazodone, mirtazapine, burpopion, tianeptine).

Furthermore, we examined the association of AD prescription with spectrum of diagnostic categories from ICD X.

Statistical analysis included parametric and non-parametric descriptive statistics, depending on the nature of data (arithmetic environment and standard deviation, mediana and inter-qwoter scope, relative frequency). Further analysis included inferential statistics methods (unifactoral analysis of variants, Students' *t* - test, Mann - Whitney's *U*-test, Pearson's, χ^2- test of indepedence, Spearman's rank correlation). Data analysis was performed by PASW Statistics18 (SPSS Inc. Chicago, IL).

Results

The choice of ADs at the University Psychiatric Clinic in Belgrade included SSRIs in 47.8% cases, TCAs in 25.3% cases and new ADs in 15.1.3% cases. ADs were prescribed to the patients with the following International Classification of Diseases (ICD) X diagnoses: unipolar depression (F 32–34) without comorbidity (46.2%) and with comorbidity (24.7%); organic mental disorder (8.7%); anxiety disorders (8.0%); psychotic disorders (7.4%); bipolar disorders (3.7%) and the other diagnoses (personality disorder and psychoactive substances abuse, 1.3%) (Figure 1).

Patients with unipolar depression (n = 137) were 50.1 ± 8.9 years old, 65% females, with high school degree in 52.6%. The first episode of depression occurred 9 years prior

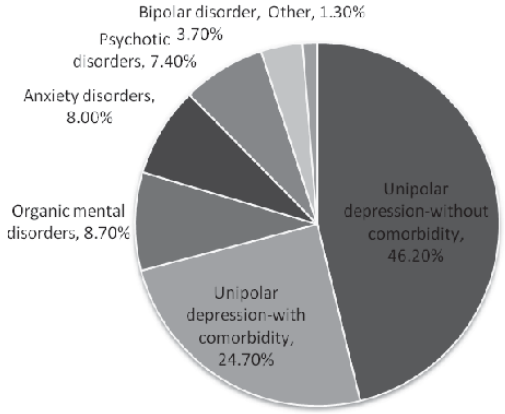

Fig. 1 – Antidepressants in different diagnostic categories – The International Classification of Diseases (ICD) X

to the current (2–15 years); the average number of hospitalizations was 2 (1–4). Patients with the other diagnoses were similar to the unipolar depression group in terms of sociodemographic and clinical parameters (47.6 ± 10.9 years of age, $p = 0.07$; females 56%, $p = 0.11$; high school 54.1%, $p = 0.96$; first diagnosis 8 years prior to the current study (3–16 years), $p = 0.70$; the number of hospitalizations 2 (1–4), $p = 0.10$)). However, the unipolar depression group was significantly different in terms of the two sociodemographic parameters: marital status (more married patients with unipolar depression, $p = 0.03$) and employment (more patients with unipolar depression were employed, $p = 0.01$), compared to the other diagnostic groups.

The frequency of the prescribed ADs is shown in Figure 2. Three most frequently prescribed ADs in monotherapy were: sertraline (20.4%), fluoxetine (13.3%) and maprotiline (11.7%).

At the same time, the most frequently prescribed groups of ADs were: SSRIs (47.8%), followed by TCAs (25.3%) and new antidepressants (15.1%).

In unipolar depression without comorbidity, one AD was prescribed in 91%, while in all other cases there were maximum of two ADs combined. The most frequently used combination of two ADs was maprotiline-clomipramine (in 33% patients). The applied dosages of ADs are shown in Table 1 (the range of recommended doses was added from the official textbook [12]).

Table 1

Antidepressant daily doses (in miligrams)

Drug	Median (mg)	25–75 percentile (mg)	Recommendations [13] (mg)
Amitriptiline	75	25–100	75–300
Clomipramine	75	50–100	75–300
Maprotiline	50	25–75	50–150
Fluoxetine	20	20–20	10–20–80
Paroxetine	20	20–40	20–60
Sertraline	50	50–100	50–200
Escitalopram	10	10–15	10–20–40
Venlafaxine	150	150–225	75–225
Mirtazapine	30	22.5–30	15–45
Moclobemide	300	300–300	150–600
Mianserine	30	30–30	30–150
Trazodone	75	50–125	150–600
Tianeptine	37.5	37.5–37.5	25–50

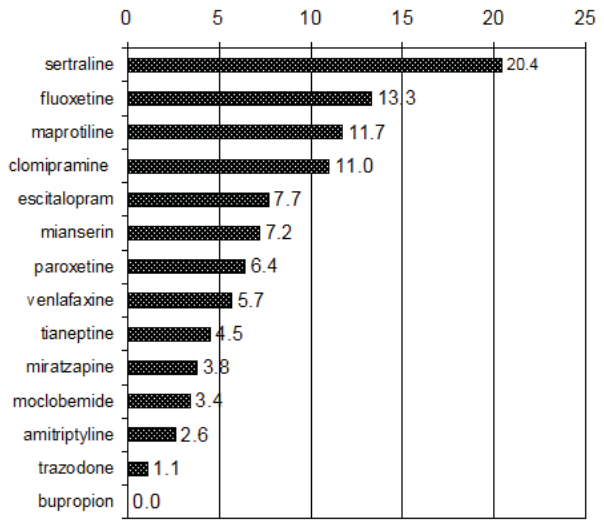

Fig. 2 – The frequency of prescribing a single antidepressant

Analysis of associations between ADs choice and the three groups of factors related to the patient, doctor and the drug, respectively showed that the three factors affected the choice of ADs in our sample of patients with unipolar depression. The first, ADs choice was associated with the severity of the actual depressive episode ($p = 0.02$); the second, economic factor had a significant effect on ADs choice (psychiatrists mostly prescribed ADs refunded in 75%-100% by the National Health Insurance, $p < 0.01$); the third, the physician's years of experience influenced significantly the choice of ADs (i.e. the doctors with more than 20 years in psychiatry were hesitating to prescribe new antidepressants; $p = 0.01$) (Figure 3).

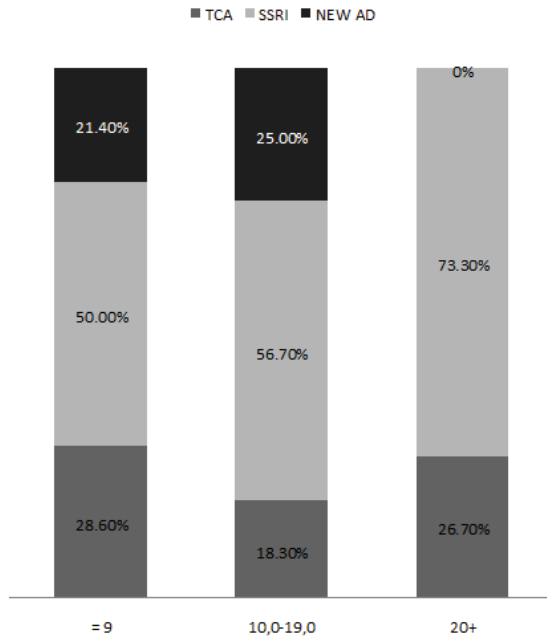

Fig. 3 – Groups of antidepressants (AD) and the frequency of prescription in relation to the psychiatrist's years of practice.

TCA – tricyclic antidepressants; SSRI – selective serotonin reuptake inhibitors

For severe depressive episodes with psychotic symptoms, TCA (60.0%) were ADs of choice, while in 73.3 % patients with moderate depression SSRI were prescribed (Figure 4). Patients' sex ($p = 0.08$), age ($p = 0.29$), employment ($p = 0.71$), duration of the illness ($p = 0.72$), suicidal attempts during the patient's history ($p = 0.89$), as well as the physician's sex ($p = 0.89$) were not associated with the choice of ADs.

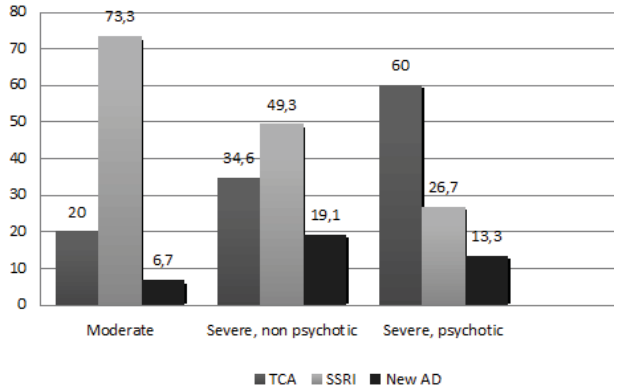

Fig. 4 – The use of antidepressants (AD) in relation to severity of depressive episode

TCA – tricyclic antidepressants; SSRI – selective serotonin reuptake inhibitors

Discussion

The current study showed that ADs were predominately prescribed to the patients diagnosed with unipolar depression, with or without comorbidity, Most frequently prescribed ADs were those from the group of SSRIs. The prescriptions combining two ADs were rare; however, when such combinations were prescribed the doses were moderate to lower in comparison to the recommended dosages. TCA ADs were most often recommended fo patients with major depression with psychotic symptoms, while SSRI were predominantly prescribed for nonpsychotic episodes of depression. In addition to the symptom profile, the most important factors in deciding which drugs to prescribe were also the position of a drug on the positive list, as well as the years of experience of the attending physician.

The results of our study are significant because they delineate the prescribing practice of the University Clinic, the major undergraduate and graduate educational institution, with the highest impact on education and training of psychopharmacologists in the country. It should be noted that the Clinic for Psychiatry experts published Guidelines for the treatment of depression in 2004 [6] as well as two editions of the textbook entitled "Antidepressants" in 2000 and 2006, respectively [12, 13], which have considerably facilitated improvement of psychiatry clinical practice in the country. Nevertheless, the current study showed that the physicians with many years of experience hesitate to prescribe new ADs. It would be of interest to investigate whether the physicians with more than 20 years of service do not prescribe new ADs due to poor experience or because they are satis-

fied with the efficacy of the older generation of drugs and have no need to widen the range. Further studies are needed to answer these questions given that the existing literature does not offer sufficient data to address this issue. Available limited data only indicate that physicians with more years of experience rarely prescribe SSRIs as their first choice of ADs; however, the years of experience have no effect on decision to prescribe new ADs (SNRIs), with an interesting result concerning the gender of the physicians [10]. Namely, the Bauer's et al. [10] study, which involved physicians from several Western European countries, showed that female physicians more often prescribe newer ADs from SNRI group than TCAs, but the authors were unable to explain such findings. The effect of the physician's or the patient's gender on the selection of ADs has not been detected in this country, although there are studies which show that SSRI drugs would be the choice of women in the generative period [14].

The most frequently prescribed ADs in our country are sertraline, fluoxetine, maprotiline and clomipramine. All four of these ADs are on the list of prescription medicine and are covered by the Obligatory Healthcare Coverage without any patient's financial obligation. Similar results were shown in a comprehensive study conducted in the Serbian Autonomic Province of Vojvodina in 2006, which showed that fluoxetine was most frequently prescribed AD and that there is a connection between the prescribing frequency and the reimbursement obtained[11]. More specifically, the fact that the coverage for the drugs from the positive list (e.g. maprotiline, clomipramine and mianserine) only extended to the obligatory participation had a considerable effect on the frequency of prescribing Ads, in general, as well as ADs from the SSRI group (e.g., sertaline, fluoxetine) for which, at the time the study, there was 10%–50% drug price reduction [11].

The effect of drug costs on the treatment choice is to be expected and was well documented in developing countries. A 2004 study by Simon et al. [15] showed that the cost of drugs in St. Petersburg hindered the treatment in 75% of patients, while a similar problem was encountered by only 24% of patients in Barcelona, or 32% in Melburn. Due to a very strong effect of the economic factor and the fact that ADs are predetermined by being listed on the aforementioned positive list, the selection and the percentage of ADs used in the treatment of depression in our country differ from the practices in the European countries. For example, in Great Britain and France, SSRIs are used in about 80% of patients and SNRIs (newer ADs) in about 10% of cases, which nearly eliminated the use of TCAs. The situation is similar in Holland, Sweden and Switzerland, while Germany is an exception, where SSRIs are used in about 31.7% of patients, while TCAs in 26.5%.[10.] With respect to the practice in the rest of the world, a 2007 multicentric study showed that "newer ADs" (i.e., SSRIs and new ADs) are less often prescribed in Japan (49%), but considerably more often in Taiwan, Singapore and China (80–70%) [9]. Despite the above-described factors, there is an increase in the use of SSRIs and new ADs in this country compared to five years ago. More specifically, in some unpublished analyses from our clinic, based on a similar methodology applied to 168 patients who were treated during 2005, TCAs were the medications of choice in 48% patients with unipolar depression, SSRIs in 39%, and new ADs in 13% of patients, respectively.

Subsequently, over the course of the following four years, it became clear that the percentage of TCA used has considerably decreased; however, it is interesting that the ADs from this group were still significantly more often prescribed for major depression with psychotic symptoms than any other ADs. According to the latest Harvard guidelines, the recommended therapy for patients suffering from major depression with psychotic symptoms is electroconvulsive therapy (ECT), followed by either TCAs, or SSRIs in combination with an antipsychotic [16]. Because ECT is used only in treatment of resistant cases, it is clear that the use of TCAs is key and correct strategy for the reduction in the mentioned symptoms. However, it should be noted that TCAs should be avoided in suicidal patients as well as if simultaneous treatment by TCAs and cardiotoxic antipsychotic is applied, in which case the choice of ADs should be an SSRI.

The fact that the doses of the prescribed ADs vary in the range of moderate to lower, which is not affected by the economic factor (the drugs covered by the health insurance are also given in smaller doses) suggests that such a therapy may be inefficient. If we compare the prescribed dosages in our and the Western Europe countries, it may be concluded that our colleagues prescribe similar dosages. However, the dosages of certain ADs prescribed by psychiatrists are considerably different from those prescribed by general practitioners: psychiatrists prescribe higher doses of amitriptiline, sertraline and venlafaxine, while higher doses of trazodone are prescribed by general practitioners [10].

In an effort to counteract an inefficient treatment, or in order to reduce side effects, combinations of two ADs are used worldwide (e.g., in Austria 25%) more often than in case of our patients (9%). The recommended combinations of ADs include the following strategies[17]: serotonergic (SSRI with trazodone), noradrenergic (TCA with bupropion) or combined strategy (venlafaxin and mirtazapine; bupropion and SSRI), respectively. Therefore, it is unclear why in the current sample the majority of combined therapies included two TCA, which, it should be pointed out, may be very risky. Given that recommended combinations of ADs include new ADs, we believe that the physicians will gain necessary skills for the application of combinations of ADs as the result of rational psychopharmacotherapy with longer use and better availability of new drugs.

Conclusion

The current study showed that economic factors, psychiatrist's years of experience and the severity of depression are the major factors that significantly effect drug choice in a particular sample. The fact that SSRIs were most frequently prescribed ADs is consistent with the practice in the majority of developed countries. These results are important because they delineate the practice of the University Clinic, the major undergraduate and graduate educational institution, with the

highest impact on education and training of psychopharmacologists in the country. We suggest that methodologically similar studies need to be conducted at the national level as an important step prior to the official introduction of national algorithms.

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Značaj patohistološkog nalaza i ekspresije Bcl-2 za prognozu i lečenje oralnog planocelularnog karcinoma

Significance of pathohistological findings and the expression of Bcl-2 in diagnosis and treatment of oral planocellular carcinoma

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Apstrakt

Uvod/Cilj. Mnogobrojna istraživanja usmerena su na detekciju i karakterizaciju različitih tumorskih markera kod oralnog planocelularnog karcinoma sa ciljem da se smanji morbiditet i mortalitet, kao i uspostavi korelacija između stepena ekspresije određenog markera i prognoze bolesti. Cilj ovog rada bio je da se utvrde patohistološke karakteristike tumorskog i peritumorskog tkiva bolesnika sa oralnim planocelularnik karcinomom, posebno stepen ekspresije markera Bcl-2, da se ukaže na značaj korelacije kliničkomorfoloških parametara, kao i na mogućnost primene ovih rezultata u kliničkoj praksi. **Metode.** Studija je obuhvatila 62 bolesnika u II i III stadijumu oralnog planocelularnog karcinoma, operisana u Klinici za maksiofacijalnu hirurgiju Vojnomedicinske akademije u Beogradu. Kod svih bolesnika određivani su stadijum bolesti, patohistološki stepen diferentovanosti tumora, a imunohistohemijskom metodom praćena je ekspresija markera Bcl-2 u peritumorskom tkivu. **Rezultati.** U analiziranoj grupi bila su 24 (39%) bolesnika sa tumorom veličine T1, 6 (9%) bolesnika sa tumorom veličine T2, a 32 (52%) bolesnika sa primarnim tumorom veličine T3. Infiltracija tumora u peritumorskom tkivu verifikovana je patohistološkom analizom okolnog vezivnog, masnog, mišićnog tkiva i kosti, koji su bili zahvaćeni resekcijom. Utvrđena je statistički visokoznačajna pozitivna korelacija između stepena ekspresije proteina Bcl-2 u peritumorskom tkivu, sa jedne strane, i histološkog gradusa tumora (ϱ = 0,468; p < 0,001), nukleusnog gradusa tumora (ϱ = 0,430; p < 0,001) i nukleocitoplazmatskog odnosa u tumorskim ćelijama (ϱ = 0,410; p = 0,001). **Zaključak.** Ekspresija Bcl-2 u kombinaciji sa patohistološkim parametrima može biti značajan prognostički faktor, pa mu treba posvetiti više pažnje u okviru multidisciplinarnog istraživanja prognoze oralnog planocelularnog karcinoma.

Ključne reči:
usta, neoplazme; karcinom, planocelularni; tumorski markeri, biološki; prognoza; lečenje, ishod; histološke tehnike; imunohistohemija.

Abstract

Background/Aim. Numerous studies were aimed to detect and characterize various tumor markers in patients with oral planocellular carcinoma in order to reduce moratlity and mobidity rates of these patients, as well as to establish the correlation between the expression of specific tumor marker and prognostic outcome. The aim of this study was to determine patohistological characteristics of tumor and peritumor tissue in patients with oral planocellular carcinoma, with special regard to the expression of Bcl-2, as well as to point out the significance of clinicomorphological correlations for clinical use. **Methods.** Sixty-two patients with oral planocellular carcinoma, stage II and III, were examined. The patients were surgically treated for this condition at the Clinic for Maxillofacial Surgery, Military Medical Academy, Belgrade. Surgical specimens were obtained from both tumor and peritumoral tissues. Patohistologic degree of tumor differentiation and the immunohistochemical expression of Bcl-2 were determinated for each specimens. **Results.** Twenty-four (39%) patients had tumor dimension T1, while six (9%) and thirty-two (52%) patients had tumor dimension T2 and T3, respectively. Patohistologic analysis of peritumor connective, fat, muscle and bone tissue samples confirmed the presence of tumor infiltration. The expression of Bcl-2 in peritumor tissue samples correlated significantly with tumor's histologic grade (ϱ = 0.468; p < 0.001), nuclear grade (ϱ = 0.430; p < 0.001) and nucleocytoplasmic ratio (ϱ = 0.410; p = 0.001). **Conclusion.** This results suggest that the expression of Bcl-2 in combination with patohistologic findings could have a prognostic value in patients with oral planocellular carcinoma.

Key words:
mouth neoplasms; carcinoma,squamous cell; tumor markers, biological; prognosis; treatment outcome; histological tehniques; immunohistochemistry

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Uvod

I pored zapaženog napretka u dijagnostici i terapiji oralnog planocelulernog karcinoma (OPK), tokom poslednje dve decenije, nije zabeleženo bolje petogodišnje preživljavanje bolesnika sa OPK. Dijagnostički i terapijski pristup zasnovan na kliničkim, morfološkim i patohistološkim elementima zaostaje za promenama prisutnim na molekularnom nivou, tako da primenjena terapija nije dovoljno efikasna. Stoga, uvek aktuelno pitanje koje se nameće istraživačima i kliničarima je iznalaženje kliničke primene novih dijagnostičkih i terapijskih pristupa u lečenju bolesnika sa OPK [1–3].

U cilju uspešnije prognoze i terapije za bolesnike sa OPK, pored patohistološke analize ispitivana je i ekspresija mnogobrojnih markera u tumorskom tkivu ovih bolesnika. Među mnogobrojnim markerima najčešće su upotrebljavani: p53, HER-2, EGFR, VEGFR, kao i Bcl-2 koji je zbog neusaglašenih podataka iz literature ocenjivan kao „enigmatičan" prognostički marker [4–6].

Zahvaćenost hirurške margine tumora tumorskim ćelijama češće se javlja kod OPK sa visokim gradusom i sa visokim stepenom maligniteta i može predstavljati direktan ili indirektan pokazatelj progresije bolesti u praćenju petogodišnjeg preživljavanja [7].

Cilj ovog rada bio je da se utvrde patohistološke karakteristike tumorskog i peritumorskog tkiva bolesnika sa OPK, proceni stepen ekspresije markera Bcl-2, da se ukaže na značaj korelacije kliničkomorfoloških parametara kao i na mogućnost primene ovih rezultata u kliničkoj praksi.

Zbog značajne uloge proteina Bcl-2 kao protoonkogena, kao i njegove centralne uloge u regulaciji apoptoze [8], naročitu pažnju posvetili smo ekspresiji ovog markera u tkivu hirurške margine kod bolesnika sa OPK. Posebno je naglašen značaj korelacije stepena ekspresije Bcl-2 u peritumorskom tkivu sa različitm patohistološim parametrima za procenu uspešnosti terapijskog pristupa.

Metode

Retrospektivno/prospektivnom studijom obuhvaćena su 62 bolesnika u II stadijumu po TNM klasifikaciji (T-tumor, N-limfni čvor, M-metastaza) (28 bolesnika) i III stadijumu (34 bolesnika) OPK, operisana u Klinici za maksilofacijalnu hirurgiju Vojnomedicinske akademije (VMA) u Beogradu, u periodu od oktobra 1995. do juna 2009. godine. Nakon operacije tumora ove regije urađena je patohistološka verifikacija tumorskog tkiva u Institutu za patologiju VMA. Posle standardne morfološke dijagnostike na tkivnim presecima fiksiranim u formalinu i ukalupljenim u parafin vršene su imunohistohemijske (IHH) analize u Laboratoriji za imunohistohemiju i eklektronsku mikroskopiju Instituta za medicinska istraživanja Univerziteta u Beogradu.

Klinički parametri relevantni za praćenje toka bolesti obuhvatali su određivanje stadijuma TNM bolesti, analizu karakteristika tumora (histološki i nukleusni gradus tumora), vrstu operacije i postoperativni tok, komplikacije posle operacije (krvarenje, infekcija i dehiscencija rane i dr).

Na redovne kontrolne preglede, u cilju utvrđivanja praćenja pojave lokalnog recidiva, kao i regionalnih i/ili udaljenih metastaza, bolesnici su dolazili po završetku lečenja (hirurgija i zračenje) jednom mesečno prve godine, svaka dva meseca u toku druge godine, svaka tri meseca u toku treće godine, a u kasnijem toku na šest meseci do godinu dana. Na svakom kontrolnom pregledu, osim kliničkog pregleda i laboratorijskih analiza krvi, svim bolesnicima urađene su i radiografske analize srca i pluća, ultrazvuk operisane regije i abdomena, a bolesnicima kod kojih je postojala sumnja na pojavu lokalnog recidiva ili regionalnih metastaza urađeni su i kompjuterizovana tomografija (CT) i nuklearna magnetna rezonanca (NMR). Kod svih bolesnika započeta je postoperativna zračna terapija u planiranom roku i svi bolesnici primili su punu terapijsku dozu zračne terapije.

Za patohistološku analizu uzorci tumorskog tkiva fiksirani su u 5% puferisanom, neutralnom formalinu i obrađeni u V.I.P. Sakura aparatu za automatsku fiksaciju, dehidraciju i prožimanje tkiva, a potom ukalupljeni u parafin. Iz parafinskih blokova tkivo je sečeno na debljinu 5–7 μm, tkivni preseci montirani na posebne visokoadherentne pločice (*Super-Frost*) i sušeni na temperaturi od 56 ºC u toku jednog sata.

Određivan je histološki i nukleusni gradus tumora, mitotski indeks, nukleocitoplazmatski odnos, opseg nekroze tumorskog tkiva, stepen infiltracije peritumorskog vezivnog, masnog, mišićnog tkiva i kosti, kao i infiltracija u peritumorske limfne sudove, vene i arterije. Stepen infiltracije peritumorskog vezivnog, masnog, mišićnog tkiva i kosti određivan je na osnovu opsega infiltracije i označavan na sledeći način: 0 – nema infiltracije; 1 – infiltracija prisutna 0,5 cm od ivice tumora; 2 – prisustvo infiltracije u 0,5–1 cm od ivice tumora; 3 – infiltracija prisutna u peritumorskom tkivu > 1cm od ivice tumora. Za markiranje tumorskih ćelija u peritumorskom tkivu korišćena su monoklonska i poliklonska antihumana antitela. Vizuelizacija ovih markera vršila se primenom visokosenzitivne i specifične IHH metode, obeležene streptavidin-biotin kompleksne metode (LSAB+-HRP kit).

U imunopatološkoj analizi materijala primenom određenih tumorskih markera (PCNA, HER–2, Bcl–2 i CD31) semikvantitativnom metodom određeni su prisustvo i broj tumorskih ćelija u peritumorskom tkivu. Na osnovu intenziteta bojenja i broja imunoreaktivnih ćelija nalaz je označen na sledeći način: 0 – nema imunoreaktivnih ćelija; 1 – slabo bojenje, retke pozitivne ćelije; 2 – umereno bojenje, mali broj pozitivnih ćelija; 3 – intenzivno bojenje, veći broj pozitivnih ćelija. U ovom radu posebna pažnja je usmerena na stepen ekspresije markera Bcl-2 u tumorskom i peritumorskom tkivu ispitivanih bolesnika.

Za praćenje postoperativnog toka bolesti korišćeni su standardni klinički pregledi za ocenu pojave recidiva tumora u 2–10-godišnjem periodu, prvenstveno kontrola prisustva potkožnih promena na delu operativnog polja kao i prisustvo lokoregionalne limfadenopatije.

Za statističku obradu podataka korišćen je komercijalni statistički paket (softver) za PC računare (SPSS for Windows; Ver.10.0). U cilju statističkog testiranja normalnosti raspodele ispitivanih obeležja korišćen je Kolmogorov-Smirnovljev test, a u cilju testiranja međugrupnih razlika

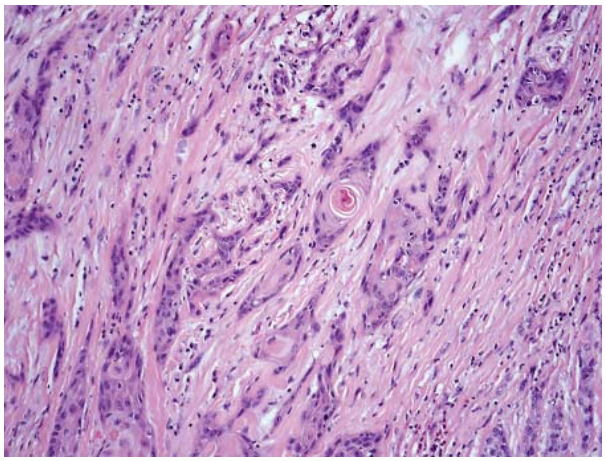
primenjeni su Kruskal-Wallis-ov test i ANOVA, u zavisnosti od tipa varijable. Korelacija određenih obeležja ispitivana je Spirmanovim koeficijentom korelacije rangova (*Spearman's rho* – ρ). Vremenski intervali bez ponovnog javljanja bolesti (dužina remisije) izračunavani su Kaplan–Meire-ovim testom i upoređivani su između grupa pomoću Log–Rank testa.

Rezultati

U retrospektivno-prospektivnoj studiji analizirana su 62 bolesnika, oba pola, koji su operisani od OPK u II stadijumu i III stadijumu bolesti (IIIa i IIIb), podeljeni u tri grupe i prikazani na onkološkom konzilijumu za maligne bolesti glave i vrata. Utvrđen je OPK II stadijuma kod 28 bolesnika i III stadijuma kod 34 bolesnika, pri čemu IIIa kod 13 bolesnika i IIIb kod 21 bolesnika. Razlika u prosečnoj starosti ispitanika, izmedu tri ispitivane grupe prema stadijumu bolesti, nije bila statistički značajna (ANOVA, F = 0,576; $p > 0,05$).

Kliničkim pregledom analizirani su parametri značajni za praćenje toka bolesti. Standardnim TNM sistemom klasifikacije klinički je određena veličina primarnog tumora (T). U analiziranoj grupi bolesnika utvrđeno je da 24 bolesnika (u II stadijumu bolesti) imaju veličinu tumora T1, 6 bolesnika (4 u stadijumu II i 2 u stadijumu IIIa) veličinu tumora T2 i da 32 bolesnika (11 u stadijumu IIIa i 21 u stadijumu IIIb) imaju veličinu primarnog tumora T3. Bolesnici sa veličinom primarnog tumora T4 nisu bili uključeni u ovu studiju (tabela 1). Prosečna izmerena veličina tumora iznosila je 3,35 cm, uz standardnu grešku 0,19 cm. Najmanji izmereni tumor imao je prečnik 0,50 cm, a najveći 6 cm, sa medijanom 4 cm. Distribucija veličine tumora nije pratila normalnu raspodelu, što je testirano Kolmogorov-Smirnovljevim testom (K-S Z = 1,780; $p = 0,004$). Veća prosečna veličina tumora utvrđena je u grupi bolesnika operisanih u IIIb stadijumu, u odnosu na bolesnike iz II i IIIa stadijuma OPK. Međutim, opisana razlika nije statistički značajna. U cilju ispitivanja korelacije klinički nalaz je upoređivan sa analizom patohistoloških karakteristika tumora.

Značajne karakteristike primarnog tumora predstavljaju histološki i nukleusni gradus, koji su utvrđeni patohistološkom analizom. Svi ispitanici su imali OPK bez udaljenih metastaza u vreme ispitivanja. Infiltracija tumora u peritumorskom tkivu verifikovana je patohistološkom analizom okolnog vezivnog (slika 1), masnog, mišićnog tkiva i kosti koji su bili zahvaćeni resekcijom, počevši od makroskopskog nalaza ivice tumora do hiruške ivice (margine).



Sl. 1 – Infiltracija tumora u vezivno tkivo (hemotoksilin-eozin, uvećanje 100)

Uočene su značajne razlike između stepena infiltracije tumorskih ćelija u peritumorskom vezivnom tkivu kod bolesnika u različitim stadijumima OPK (tabela 2).

Imunohistohemijska analiza ekspresije proteina Bcl-2 pokazala je slabo imunoreaktivno bojenje u tumorskim ćelijama kod 25 bolesnika, od kojih je 11 bilo u stadijumu II, 5 u IIIa i 9 u stadijumu bolesti IIIb (tabela 3) (slika 2).

Tabela 1

Veličina tumora i stadijum bolesti kod obolelih od planocelularnog karcinoma

Veličina tumora	Stadijum II	Stadijum III			Ukupno
		IIIa		IIIb	
T1	24	0		0	24
T2	4	2		0	6
T3	0	11		21	32
Ukupno	28	13	34	21	62

T1 – tumor prečnika do 2 cm, T2 - tumor prečnika 2–4 cm; T3 - tumor veći od 4 cm

Tabela 2

Infiltracija tumora u peritumorsko vezivno tkivo i stadijum bolesti kod obolelih od oralnog planocelularnog karcinoma

Infiltracija tumora u vezivno tkivo	Stadijum II	Stadijum III			Ukupno
		IIIa		IIIb	
VT-0	0	0		0	0
VT-1	26	5		5	36
VT-2	2	6		13	21
VT-3	0	2		3	5
Ukupno	28	13	34	21	62

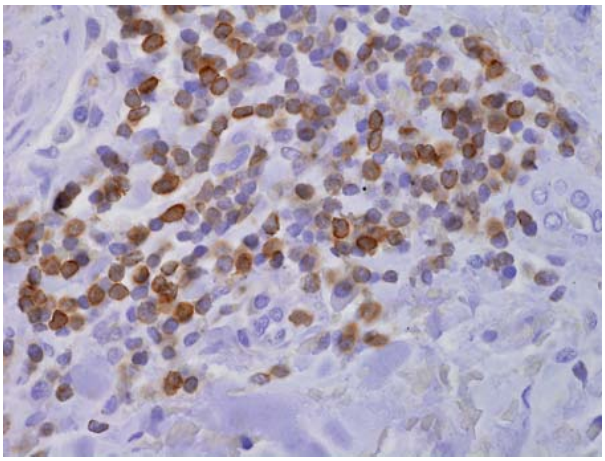
VT-0 – nema infiltracije u vezivno tkivo; VT-1 - infiltracija u vezivno tkivo 0,5 cm od ivice tumora;
VT-2 - prisustvo infiltracije u 0,5–1 cm od ivice tumora;
VT-3 - – infiltracija prisutna u vezivnom tkivu > 1 cm od ivice tumora

Tabela 3

Ekspresija Bcl-2 imunoreaktivnih ćelija u peritumorskom tkivu i stadijum bolesti kod obolelih od plancelularnog karcinoma

Bcl-2	Stadijum II	Stadijum III		Ukupno
		IIIa	IIIb	
Bcl-2-0	17	8	12	37
Bcl-2-1	11	5	9	25
Bcl-2-2	0	0	0	0
Bcl-2-3	0	0	0	0
Ukupno	28	13 34	21	62

Bcl-2-0-nema imunoreaktivnih ćelija; Bcl-2-1 – slabo bojenje, retke pozitivne ćelije;
Bcl2-2 – umereno bojenje, mali broj pozitivnih ćelija; Bcl-3 – intenzivno bojenje, veći broj pozitivnih ćelija



Sl. 2 – Tumorske Bcl-2-pozitivne ćelije u vezivnom tkivu (imunohistoohemijsko bojenje, uvećanje 400x)

Razlike u stepenu ekspresije proteina bcl-2 u ćelijama peritumorskog tkiva, u odnosu na stadijum bolesti, nisu bile statistički značajne, što je potvrđeno primenom Kruskal-Wallis-ovog testa ($\chi 2 = 0,086$; $p > 0,05$).

Karakteristike značajne za praćenje toka bolesti podrazumevale su analizu povezanosti parametara koji bi mogli da utiču na tok već dijagnostikovane maligne bolesti. Kao početni parametar određivan je preoperativni stadijum bolesti za svakog bolesnika. Primenjujući TNM sistem klasifikacije, 28 bolesnika bilo je u II, a 34 bolesnika u III stadijumu bolesti (13 u stadijumu IIIa i 21 u stadijumu IIIb). Analizirana je distribucija parametara karakterističnih za primarni tumor u različitim stadijumima bolesti u cilju utvrđivanja njihove učestalosti koja bi mogla biti pokazatelj proširenosti tumora i dužine ukupnog preživljavanja. Među ovim parametrima poseban prediktivni značaj ima veličina (prečnik) primarnog tumora, koja je statistički bila značajno manja kod bolesnika u stadijumu II nego kod bolesnika u stadijumu bolesti IIIa i IIIb (tabela 1).

Primenom Spirmanovog koeficijenta korelacije rangova utvrđena je statistički visokoznačajna pozitivna korelacija između histološkog gradusa i stepena infiltracije peritumorskog vezivnog ($\rho = 0,636$; $p < 0,001$) i mišićnog tkiva ($\rho = 0,646$; $p < 0,001$). Statistički značajna korelacija utvrđena je i između nuklearnog gradusa, sa jedne strane, i stepena infiltracije peritumorskog vezivnog ($\rho = 0,629$; $p < 0,001$) i mišićnog tkiva ($\rho = 0,661$; $p < 0,001$), sa druge strane. Takođe, pokazano je da je stepen infiltracije peritumorskog vezivnog, masnog i mišićnog tkiva u pozitivnoj korelaciji sa nukleocoitoplazmatskim odnosom tumorskih ćelija (vezivno tkivo, $\rho = 0,406$; $p = 0,001$; masno tkivo, $\rho = 0,312$; $p = 0,014$; mišićno tkivo, $\rho = 0,462$; $p < 0,001$). Utvrđena je statistički visokoznačajna pozitivna korelacija između stepena ekspresije proteina Bcl-2, sa jedne strane, i histološkog gradusa tumora ($\rho = 0,468$; $p < 0,001$), nukleusnog gradusa tumora ($\rho = 0,430$; $p < 0,001$) i nukleocitoplazmatskog odnosa u tumorskim ćelijama ($\rho = 0,410$; $p = 0,001$).

Diskusija

O uzrocima i kliničkoj slici planocelularnog karcinoma jezika i poda usta, uključujući i prognozu zasnovanu na TNM sistemu određivanja stadijuma bolesti i značaju histološkog nalaza, danas se mnogo zna. To, međutim, nije dovelo do značajnijeg poboljšanja u lečenju bolesnika sa OPK, pa je pažnja istraživača sve više usmerena ka rasvetljavanju molekularne osnove porekla ovog tipa karcinoma. To bi doprinelo utvrđivanju biomarkera za ranu detekciju, kao i prognostičkih i prediktivnih markera koji bi pomogli u donošenju odluke oko izbora efikasnijeg modaliteta lečenja[1, 2, 9, 10].

Oralni planocelularni karcinom nastaje kao krajnji stadijum višestepenog procesa kancerogeneze, koji je vođen genetskim i epigenetskim oštećenjima, uglavnom uzrokovanih delovanjem kancerogenih agenasa. Ključnu ulogu u ovom procesu imaju dve klase gena: protoonkogeni i tumor supresor geni, koji su neophodni za kontrolu procesa proliferacije, diferencijacije i smrti ćelije.

U cilju istraživanja metastatskog potencijala tumora često je analiziran niz genetskih parametara p53, Bcl-2, HER-2, EGF, VEGF koji su poređeni sa više histopatoloških parametara (prisustvo keratinizacije, nukleusni pleomorfizam, broj mitoza, način i stepen invazije i infiltracija limfocitima i dr) [6, 11, 12].

Mnogobrojna istraživanja usmerena su na otkrivanje i utvrđivanje osobina markera rasta kod OPK sa ciljem da se snizi morbiditet i mortalitet, kao i uspostavi korelacija između stepena ekspresije određenog markera i prognoze bolesti. Nova saznanja o molekularnim mehanizmima razvoja tumora podstakla su pokušaje da se molekularna genetika iskoristi za rešavanje nekoliko bitnih kliničkih problema, kao što su rana detekcija premaligne i maligne lezije, rano otkrivanje lokalnog recidiva i udaljenog širenja, rana prevencija sekundarnog tumora i, posebno, selekcija bolesnika za specifično lečenje [1, 10, 13–15].

Dok pojedine oblasti onkologije već godinama koriste prednosti specifičnih tumorskih markera, za OPK se još ne naziru prava rešenja. Najviše pažnje posvećeno je izučavanju gena p53, bilo izolovano ili u panelu sa drugim potencijalnim markerima, kao što su ciklin D1, TGF, EGF, VEGF, E-kadherin i kolagen VII15.

Osnovni morfološki elementi pomoću kojih se procenjuje stepen maligniteta, kao što je histološki gradus, nukleusni gradus, mitotski indeks, nukleocitoplazmatski odnos, sa našim rezultatima, sa jedne strane, čine statistički značajnu korelaciju prema stepenu infiltracije tumorskog tkiva u analiziranoj margini, kao i u vezivnom, masnom i mišićnom tkivu.

Patogeneza i stadijumi bolesti, s jedne, a odgovor na lečenje i prognoza, s druge strane, procenjuju se stopom petogodišnjeg preživljavanja, pri čemu se ovaj period meri od momenta dijagnostikovanja maligne bolesti, a varira zavisno od lokalizacije. Postoje mnoge studije koje su pokazale razliku u stopi preživljavanja obolelih od oralnog karcinoma. Rezultati pojedinih studija pokazuju slabo preživljavanje bolesnika kojima je dijagnostikovan OPK sa povećanom ekspresijom Bcl-2, a kod kojih je primenjena samo hirurška terapija. Nešto bolju prognozu imali su bolesnici kod kojih je, pored hirurške terapije, primenjena i radioterapija [16–18].

Oralni planocelularni karcinom ne pokazuje značajna antigena svojstva. U današnje vreme, kada se zna za više od sto kako dijagnostičkih, tako i prognostičkih parametara malignih tumora, nema specifičnih markera za OPK. Naš izbor IHH markera (PCNA, HER-2, CD31, Bcl-2) učinjen je na osnovu mogućnosti, ali i kao procena korišćenja nove kombinacije markera, za razliku od često primenjivanih IHH markera kod OPK, kao što su p53, EGF, VGF i dr.

Za sada je jasno da ne postoji nijedan specifičan marker za OPK. Podaci u literaturi obiluju različitim nalazima. Do sada, najviše pažnje posvećeno je izučavanju ekspresije gena p53, bilo izolovano ili u panelu sa drugim potencijalnim markerima, kao što su ciklin D1, TGF, EGF, VEGF, E-kaderin, kolagen VII [4, 19, 20.]

Jedan od najčešće primenjivanih IHH markera u dosadašnjim istraživanjima OPK je p53. U pojedinim oblastima onkologije već godinama koriste se prednosti specifičnih tumorskih markera, dok se za OPK još uvek ne naziru prava rešenja.

Rezultati dosadašnjih ispitivanja parametara proliferacije pokazuju pozitivnu korelaciju imunohistohemijski detektovanih proteinskih produkata PCNA, Ki-67 sa gradusom tumora i kliničkim stadijumom OPK. Za identifikaciju endotelnih ćelija u OPK korisni su CD31, VEGF i dr. [19, 21–23].

Još uvek je nejasan prognostički značaj markera Bcl-2 kod bolesnika sa OPK koji su hirurški lečeni. Studija Yuen et al [15] obuhvatila je imunohistohemijsku analizu tkiva 73 planocelularna karcinoma jezika na prisustvo ekspresije Bcl-2 i urađena je korelacija sa kliničkopatološkim nalazima. Od 73 tumora, ekspresija Bcl-2 bila je prisutna u 11% uzoraka. U ovoj studiji nije utvrđena korelacija između ekspresije Bcl-2, s jedne i gradusa tumora, stadijuma tumora i prisustva metastaza u limfnim nodusima, s druge strane. Takođe, nije utvrđen prognostički značaj u ishodu preživljavanja navedenih bolesnika koji su hirurški lečeni, što ukazuje na to da određivanje stepena ekspresije Bcl-2 ima minorni značaj kod bolesnika sa planocelularnim karcinomom jezika [15, 24, 25].

Geni čiji proteinski produkti predstavljaju regulatore procesa apoptoze imaju kritičnu ulogu u razvoju i progresiji OPK. Rezultati nekih studija pokazali su da ekspresija onkoproteina Bcl-2 predstavlja nezavisan prognostički paramentar i pokazuje negativnu korelaciju sa ekspresijom mutiranog p53 u istom stadijumu karcinoma [17].

Da bi uradili predloženu analizu tkiva u hirurškoj margini OPK opredelili smo se, pored detaljnog patohistološkog pregleda, za nama dostupne IHH markere, koji su značajni, kako u prepoznavanju maligne ćelije, tako i za ocenu prognoze i procesa širenja maligne bolesti. Upotrebljeni su PCNA, Bcl-2, HER-2 i CD31. Naši rezultati ukazuju na to da je ekspresija ispitivanih markera u peritumorskom tkivu i vaskularnim elementima tumora visokog stepena maligniteta u korelaciji sa lošom prognozom i kraćim periodom preživljavanja bolesnika nakon operacije

Ekspresije proteina Bcl-2 bila je zastupljena u 40% ispitivanih tumora. Neusaglašenost podataka iz literature koji se odnose na ekspresiju proteina bcl-2 u OPK može biti posledica različitih lokacija ovih maligniteta u oralnoj duplji. Kod laringealnih karcinoma pokazano je da je ekspresija Bcl-2 u statistički značajnoj pozitivnoj korelaciji sa gradusom tumora, odnosno da je znatno viši stepen ekspresije ovog proteina zabeležen u tumorima višeg stepena maligniteta. Za razliku od laringealnih karcinoma, dosadašnja ispitivanja ekspresije Bcl-2 u OPK nisu potvrdila ovakav nalaz [24, 25].

Zaključak

Dobijeni rezultati ukazuju na to da se genetski markeri mogu koristiti za rešavanje značajnih kliničkih problema, kao što su rano otkrivanje premalignih i malignih lezija, rano otkrivanje lokalnog recidiva i udaljenog širenja, rana prevencija sekundarnog tumora i posebno selekcija bolesnika za primenu specifičnog terapijskog protokola.

Saznanja u ovom radu proistekla su iz jednog novog pristupa izučavanju OPK gde je primenjena korelacija kliničkog praćenja hirurškog i zračnog lečenja sa mnogobrojnim patohistološkim analizama i imunohistohemijskom ekspresijom markera koji su ranije manje ispitivani, a posebno sa morfološkim elemenatima u tkivima koja ulaze u sastav hirurške margine OPK.

Rezultati ukazuju na to da Bcl-2 u kombinaciji sa određenim patohistološkim parametrima može da bude značajan prognostički faktor, pa mu treba posvetiti više pažnje u okviru multidisciplinarnog istraživanja prognoze OPK. Bolje razumevanje uloge pojedinih markera i određivanje prisustva malignih ćelija u različitim tkivima hirurške margine treba da doprinese poboljšanju hirurškog lečenja OPK.

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Microleakage, adaptation ability and clinical efficacy of two fluoride releasing fissure sealants

Ispitivanje mikrocurenja, površinske adaptacije i kliničke efikasnosti dva zalivača fisura sa sposobnošću otpuštanja fluorida

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Abstract

Background/Aim. Retention of fissure sealants and good adaptation to enamel are essential for their success. Fluoride releasing resin-based materials are widely accepted for pit and fissure sealing, but newly designed glass ionomers can serve as a good alternative. The aim of this study was to evaluate microleakage and sealing ability *in vitro,* and to clinically assess two fluoride releasing fissure sealants. **Methods.** The sample for experimental study consisted of 20 freshly extracted intact human third molars, divided in two experimental groups according to the sealing material: fluoride releasing resin-based (Heliosel F) and glass ionomer (Fuji Triage) material. Digital images and scanning electron microscope were used to assess microleakage and adaptation ability. Sample for clinical study consisted of 60 children, aged 6–8 years, with high caries risk, divided in two groups according to the sealant material. Fissure sealant was applied to all erupted, caries-free first permanent molars. Sealants were evaluated after 3, 6 and 12 months using modified Ryge criteria for retention, marginal adaptation, colour match, surface smoothness and caries. **Results.** Microleakage was detected in more than half of the specimen, without significant differences between the two groups ($p > 0.05$). Both materials exhibited acceptable sealing ability. Complete retention at the end of the observation period was 81.8% for resin-based, and 21.1% for glass-ionomer fissure sealant ($p < 0.001$). The presence of caries in sealed molars has been detected in one patient in both groups. During the 12-month observation period, Helioseal F demonstrated better retention, marginal adaptation and surface smoothness ($p < 0.001$). There were no differences between the two materials regarding caries and color match ($p > 0.05$). **Conclusion.** Both tested materials demonstrate satisfactory clinical and caries prophylactic characteristics that justify their use in contemporary preventive dentistry.

Key words:
pit and fissure sealants; fluorides; ion exchange resins; glass; sensitivity and specificity; child.

Apstrakt

Uvod/Cilj. Retencija zalivača fisura i dobro prilagođavanje površini gleđi prestavljaju suštinu njihove uspešnosti. Materijali na bazi smole koji emituju fluorid široko su prihvaćeni za zalivanje jamica i fisura, za koje su novi glasjonomeri dobra alternativa. Cilj ispitivanja bio je određivanje mikrocurenja, površinske adaptacije i kliničke efikasnosti dva zalivača fisura sa sposobnošću otpuštanja fluorida. **Metode.** U eksperimentu je korišćeno 20 sveže ekstrahovanih trećih molara podeljenih u dve grupe u zavisnosti od postavljenog materijala: kompozitni (Helioseal F) i glasjonomerni (Fuji Triage) zalivač jamica i fisura. Za procenu mikrocurenja i površinske adaptacije korišćene su digitalne fotografije i skening elektronski mikroskop. U kliničkoj studiji uzorak je činilo 60 dece visokog rizika od nastanka karijesa, uzrasta 6–8 godina, podeljenih u dve grupe u zavisnosti od materijala za zalivanje fisura. Za evaluaciju, nakon 3, 6 i 12 meseci, korišćeni su modifikovani Ryge-ovi kriterijumi za retenciju, marginalnu adaptaciju, ivičnu prebojenost, površinsku hrapavost i prisustvo karijesa. **Rezultati.** Fenomen mikrocurenja detektovan je na više od polovine eksperimentalnih zuba bez statistički značajne razlike između ispitivanih grupa ($p > 0,05$). Oba materijala pokazala su dobru adaptaciju uz zidove fisura. Potpuna retencija na kraju opservacionog perioda iznosila je 81,8% za kompozitni i 21,1% za glasjonomerni zalivač ($p < 0,001$). Karijes je detektovan kod jednog ispitanika u obema grupama. Heliosel F pokazao je bolje rezultate u pogledu retencije, marginalne adaptacije i površinske hrapavosti u odnosu na Fuji Triage ($p < 0,001$). Što se tiče karijesa i ivične prebojenosti, nije bilo razlike između ispitivanih materijala ($p > 0,05$). **Zaključak.** Ispitivani materijali pokazuju zadovoljavajuće profilaktičke karakteristike u nastanku karijesa.

Ključne reči:
zub, zalivači jamica i fisura; fluoridi; smole, jonoizmenjivačke; staklo; osetljivost i specifičnost; deca.

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Introduction

The prevalence of dental caries has been decreased during the last decades, but it is still a widespread disease [1]. Effects of caries preventive measures are greater on smooth surfaces, while occlusal caries remains a problem. It has been shown that a carious lesion most frequently occurs in pits and fissures of occlusal surfaces [2], primarily due to their specific anatomy [3], which is considered to be an ideal site for the retention of bacteria and food remnants rendering mechanical means of debridement inaccessible [4].

Sealing pits and fissures is considered to be an effective way of preventing caries development [5]. A fissure sealant is a material that is placed in pits and fissures of teeth in order to prevent or arrest the development of dental caries. Any primary or permanent tooth judged at risk would benefit from sealant application [6].

Today, there is a wide spectrum of available sealing materials. These materials differ according to the base material, the method of polymerisation and weather or not they contain fluoride. Resin sealants are bonded to the underlying enamel by the use of the acid etch technique. Their caries preventive effect is based on the establishment of a tight seal which prevents leakage of nutrients to the microflora in the deeper parts of the fissure. Glass ionomer cements are mainly recommended for pits and fissures sealing for two reasons. First, they are less susceptible to moisture which allows their use in noncooperable children or in partially erupted teeth where isolation could be a problem [7] and secondly, due to their potential to act as a fluoride reservoir making enamel more resistant to demineralisation [8].

Microleakage is defined as the passage of bacteria, fluids, molecules, and ions between the teeth and the sealing material. Microleakage is considered as the main problem with direct restorative procedures and one of the main reasons for restoration failure [9]. A dental sealant is successful only if it firmly adheres to the enamel surface, and protects pits and fissures from the oral environment.

The aim of this study was to evaluate microleakage and sealing ability *in vitro*, and to clinically assess two fluoride-releasing fissure sealants.

Methods

Experimental study

Twenty intact third molars extracted form orthodontic or surgical reasons were used in this study. Teeth were stored in the same bottle in distilled water at +4°C for a period not longer than 1 month. Specimen was randomly divided into two groups (n = 10): resin-based fluoride-releasing fissure sealant, Helioseal F (Ivoclar Vivadent AG, Schaan, Liechtenstein), and glass ionomer sealant, Fuji Triage (GC Int., Tokyo, Japan). The materials used in this study were prepared according to the manufacturers' instructions. For resin-based sealant, enamel was etched with 37% phosphoric acid gel (Total Etch, Ivoclar Vivadent AG) for 20 s. Sealant was applied and polymerized utilizing a visible light for 40 s after a 20 s interval. For glass ionomer cement, enamel was conditioned with 10% polyacrilic acid for 20 s (GC Dentin Conditioner, GC Int.), the material was applied and coated with varnish (GC Fuji Coat LC, GC Int.) which was light cured for 10 s to protect material from moisture and desiccation. After application of the sealant, teeth were stored in distilled water at +4°C for one week. During this period, teeth were thermocycled at 5°–7°C, 35°–37°C, and 55°–57°C for 300 cycles, with a dwell time of 30 s. Teeth were coated with nail varnish, except 1 mm around the sealant, and subsequently immersed in 5% methylene blue for 24 hours. Each tooth was then sectioned at 3 sites in the buccolingual plane using a water-cooled diamond-impregnated law speed saw (Isomet Low Speed Saw, Buehler; Lake Bluff, IL, USA), yielding 6 sectioned surfaces per sample for analysis.

Digital images were used to assess microleakage. Photographs were made using a camera (Olympus SP565, Tokyo, Japan) at 10 × magnification (Figure 1). One blinded examiner evaluated depth of dye penetration in each section. The scoring system [10] is described in Table 1.

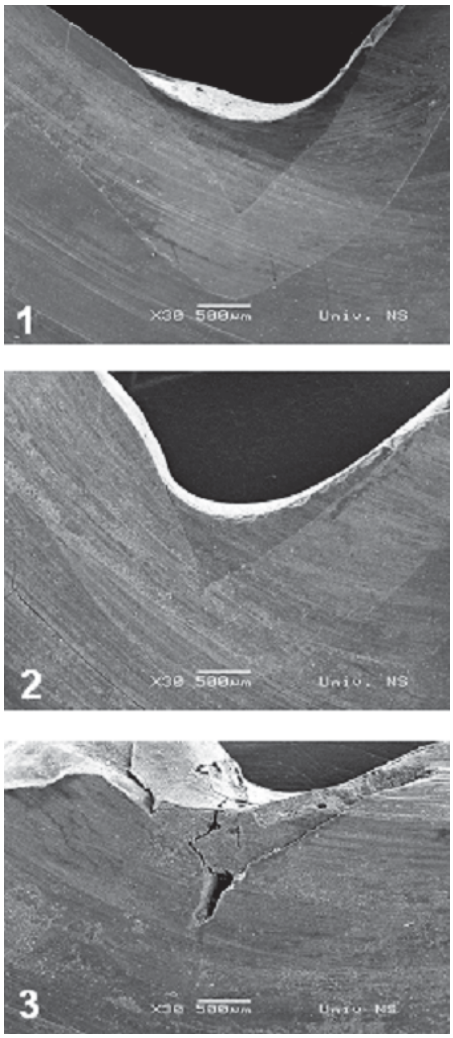

Fig. 1 – Scoring for adaptation ability

Adaptation ability was evaluated using scanning electron microscopy (SEM). The specimens were mounted on aluminium stubs, sputter-coated with gold (Bal-Tec SCD 005

Table 1

Criteria for evaluation in the experimental study

Microleakage		Adaptation ability	
Score	Description	Score	Definition
0	No dye penetration		
1	Dye penetration restricted to the outer half of the sealant	1 – good	Complete adaptation to all fissure walls
2	Dye penetration to the inner half of the sealant	2 – fair	One minor failure of adaptation
3	Dye penetration into the underlying fissure	3 – poor	Major failure of adaptation

Sputter Coater; Balzers, Liechtenstein) and than examined with SEM (JEOL JSM-6460LV, JEOL Industries; Tokyo, Japan). To standardise the microscopic observation, micrographs of the fissures were taken at magnification of 30 × (Figure 2). Scoring for adaptation ability [11] is described in Table 1.

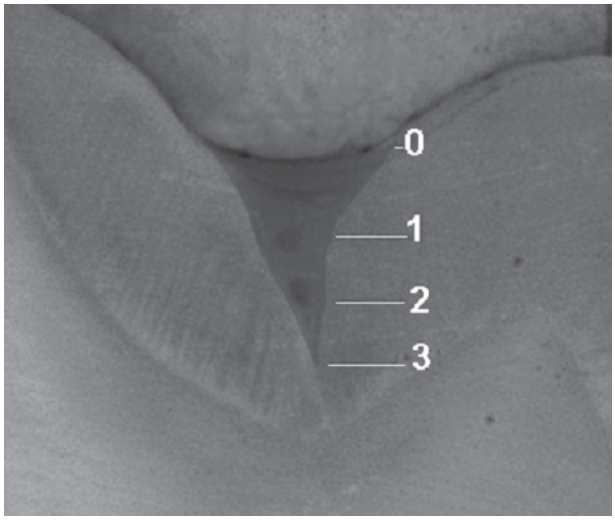

Fig. 2 – Scoring for microleakage

Clinical study

This was a prospective clinical trial with a 12-month observation period. Patients were treated at the Dentistry Clinic of Vojvodina, the University of Novi Sad, and at the Clinic for Paediatric and Preventive Dentistry, the University of Belgrade. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the local ethics committee.

The sample was composed of 60 children, aged 6–7 years, with at least one active caries lesion, restored tooth or primary tooth extracted due to the caries complications. All patients appeared for a regular dental examination when it was determined whether they met the inclusion criteria. The included patients had at least two recently erupted permanent molars with sound pits and fissures. Teeth with an obvious cavity, with a restoration or a sealant completely or partially presented in the fissure system were excluded from the study. The children and the parents were precisely informed on the purpose of the investigation, clinical procedures to be performed, and the possible benefits and potential risks involved. Informed parent consents were obtained in writing prior to the childrens participation in the study. Informed assents were obtained from the children.

The children were randomly divided into two groups (n = 30) according to the sealing material. The sealing materials, Helioseal F and Fuji Triage were placed according to manufacturers' instructions.

Two clinicians that were standardized for fissure sealing performed the sealing procedure. Two examiners evaluated all sealants. Ten percent of each investigator's sample was randomly assessed by another investigator to check inter-examiner reliability. Kappa inter-examiner reliability score was 0.93. Sealants were evaluated using a dental mirror and an explorer after 3, 6 and 12 months following the modified Ryge's criteria [12] for sealant retention, marginal adaptation, color match, surface smoothness and the presence of caries (Table 2).

Table 2

Modified Ryge criteria for clinical sealant evaluation

Criterion	Score	Definition
Retention	A	Sealant completely present
	B	Partial loss of sealing material
	C	Complete loss of sealing material
Marginal adaptation	A	Sealant is continuous with adjacent tooth structure
	B	Visible evidence of crevice formation that an explorer will penetrate
Colour match	A	Visually undetectable
	B	Mismatch in colour outside acceptable range
Surface smoothness	A	As smooth as natural adjacent tooth structure
	B	Not as smooth as natural tooth structure but not pitted
	C	Not as smooth as natural tooth structure and pitted
Caries	A	Caries free tooth
	B	Caries present

The χ^2 test was used to asses differences between the tested materials and the level of significance was set at $p < 0.001$.

Results

Experimental study

Regarding the adaptation ability, there were no statistically significant differences between glass ionomer and resin-based fissure sealants ($p > 0.05$; Table 3).

Some extent of microleakage was detected in more than 70% of the complete specimen, but without statistically significant differences between the tested materials ($p > 0.05$; Table 3).

Table 3

Adaptation ability and microleakage of fissure sealants

Score	Sealants		
	Fuji triage n (%)	Helioseal F n (%)	Statistical analysis
Adaptation ability			
good	7 (11.6)	8 (13.3)	
fair	40 (66.7)	38 (63.3)	$\chi^2 = 0.76$
poor	13 (21.7)	14 (23.4)	$p > 0.05$
Microleakage			
0	26 (43.3)	23 (38.3)	
1	15 (25.0)	17 (28.4)	$\chi^2 = 4.63$
2	13 (21.7)	11 (18.3)	$p > 0.05$
3	6 (10.0)	9 (15.0)	

Clinical study

The results of the clinical examination of resin-based and glass ionomer fissure sealants are shown in Table 4. Regarding retention, resin-based fissure sealant exhibited higher retention rate at control examinations after 3, 6 and 12 months in comparison with glass ionomer ($p < 0.001$). In addition, better scores were recorded for Helioseal F when surface smoothness was analysed ($p < 0.001$). Regarding color match, marginal adaptation and caries, there were no statistically significant differences between the tested materials during the observation period ($p > 0.05$).

Materials in this study are representative for their groups. Resin-based sealant with fluoride (Helioseal F) is an improved descendant of previous resin-based sealants. Glass ionomer sealant used in this study (Fuji Triage) is the only glass ionomer material for fissure protection available in the market. It is claimed to have greater fluoride release compared with other glass ionomer materials, as well as the highest recharge capacity [13].

The efficacy of pit and fissure sealants depends on their ability to achieve adequate bonding with conditioned enamel. Both glass ionomer and resin based fissure sealants interact with enamel surface during bonding procedure and adaptation to fissure walls can affect clinical performances of a placed material. In the present study adaptation ability was evaluated with SEM. Because of its magnification and depth of focus, SEM provides visual observation of the adaptation of sealing material to enamel walls through the whole fissure system.

SEM analysis showed that both tested materials demonstrated satisfactory adaptation ability. In the glass ionomer specimen group, the presence of cohesive failures was recorded. Even though cohesive failures were seen in all glass ionomer specimens and detachment of sealants occurred, there was still a continuous layer of a sealant covering the enamel. Fracture of the sealant above this layer probably occurred as a result of a low cohesive strength of glass ionomers, and invasive experimental preparation procedures. Similar findings were described by Birkenfeld et al. [14]. In the Helioseal F group no cohesive failures were observed, as the material is resin-based, and unlike glass ionomer, less desiccation sensitive with higher cohesive strength.

Many studies demonstrate that there is no material that could hermetically seal pits and fissures and prevent gap formation and subsequent microleakage. The most likely explanation for the gap formation is difference in thermal expansion between sealing material and the tooth structure [15]. Coefficients of thermal expansion for sealing materials are 2–4 times greater when compared with enamel [16]. Daily temperature fluctuation in the oral environment can result in gap formation and bacterial penetration through sealant/enamel interface. Based upon this explanation, techniques of thermal cycling and cycling under loading are frequently used to determine the extent of microleakage. In the present study specimens were thermocycled between 4°C and 55°C.

The use of organic dyes as tracers is the most common method for microleakage assessment *in vitro*. In the present

Table 4

Clinical evaluation of fissure sealants according to the modified Ryge criteria*

Sealants		Retention			Marginal adaptation		Color match		Surface smoothness			Caries	
	Evaluation period months	A *n* (%)	B *n* (%)	C *n* (%)	A *n* (%)	B *n* (%)	A *n* (%)	B *n* (%)	A *n* (%)	B *n* (%)	C *n* (%)	A *n* (%)	B *n* (%)
Fuji Triage	3	48 (64)	26 (35)	1 (1)	61 (81)	14 (19)	75 (100)	–	30 (40)	45 (60)	–	75 (100)	–
	6	31 (46)	31 (46)	5 (8)	40 (60)	27 (40(	67 (100)	–	17 (25)	50 (75)	–	67 (100)	–
	12	12 (21)	39 (69)	6 (10)	23 (40)	34 (60)	54 (95)	3 (5)	2 (3)	55 (96)	–	55 (97)	2 (3)
Helioseal F	3	73 (95)	4 (5)	–	73 (95)	4 (5)	77 (100)	–	75 (97)	2 (3)	–	77 (100)	–
	6	63 (88)	7 (10)	1 (2)	63 (89)	8 (11)	71 (100)	–	69 (97)	2 (3)	–	71 (100)	–
	12	45 (82)	7 (13)	3 (5)	46 (84)	9 (16)	53 (96)	2 (4)	48 (87)	7 (13)	–	54 (98)	

***For explanation see Table 2**

Discussion

Resin-based fluoride-releasing sealants have been developed in effort to add therapeutical and preventive effect of fluoride to a material with excellent mechanical and retentive characteristics. Application of glass ionomers as fissure sealants is based upon their ability to form chemical bond with tooth tissues and continuing fluoride release.

study specimens were stored in methylene blue for 24 h, according to the methodology used in the studies by Hatibovic et al. [17] and Birkenfeld at al. [14], and microleakage was scored according to the level of leakage at the sealant/enamel interface. All specimens in the present investigation showed some amount of microleakage. This finding support reports by Theodoridou-Pahini et al. [15] and Borem and Fiegel [18] who stated that microleakage can be expected in all restorative materials.

Although it is clear that there is no sealing material, application technique or sealing procedure that can prevent microleakage [17, 19, 20], results of the studies in which glass ionomer and resin-based fissure sealants are compared are not uniform. According to some reports [14, 21], higher extent of microleakge was observed under glass ionomer sealant, which is attributed to the solubility of the material. Pardi et al. [22] showed no differences between conventional glass ionomer, resin-modified glass ionomer and resin-based fissure sealants.

With the improvement of contemporary materials for pit and fissure sealing, clinical evaluation that comprises only data regarding retention and caries are considered insufficient. That is the reason why in this study modified Ryge [12] criteria were used.

The results of the present clinical evaluation clearly confirm that resin-based sealant possess superior retention in comparison with glass ionomer material. In a study with two-cohort design, Simonsen [23] found complete retention in 27.6% of sealed first permanent molars with caries reduction rate of 52% 15 years after a single application. Raadal et al. [24] and Gandini et al. [25] reported complete retention rate after two years of 97% and 66%, respectively. In a study by Vrbic [26], 95.8% of permanent molars and 91.5% of premolars treated with Helioseal F were completely sealed after 3 years. However, older participants were included in that study, and this is probably the explanation for such a high retention rate.

The longevity of glass ionomer cements as sealants is significantly lower when compared with resin-based sealants [27]. Findings on use of conventional glass-ionomer fissure sealants [24, 28], as well as resin-modified glass ionomers [29] uniformly demonstrate their lower retention rates in comparison with resin-based fissure sealants. The results from the present investigation completely correspond to these findings.

Despite higher clinical loss, glass ionomer sealant showed equal caries preventive effect as resin-based sealant. Some studies verified no differences in caries incidence or even better preventive effects for glass ionomer sealing materials, even though their retention rate was lower than for resin-based sealants [30–32]. Nevertheless, other studies found better retention and caries preventive effect of resin-based fissure sealants [33, 34].

A relevant factor that should be considered when glass ionomer material is studied as a fissure sealant is that even after it has been clinically lost, small amounts of sealant are left at the bottom of the fissure and continue to release fluoride [8], providing another kind of occlusal protection.

For both tested material, the absence of marginal discoloration was observed during the entire observation period. Regarding marginal adaptation and surface smoothness, resin-based material showed superior results when compared to glass ionomer. These results completely correspond with the literature [35].

Conclusion

Resin-based and glass ionomer fissure sealant demonstrate satisfactory sealing ability. None of the tested materials could prevent dye penetration, suggesting that microleakage still can occur in real clinical situations. Although resin-based fissure sealant demonstrates better retention, both materials are equally effective in caries prevention, and could be recommended as materials of choice for pits and fissure sealing procedure.

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Razlike u težini depresije i učestalosti recidiva kod opijatskih zavisnika lečenih metadonom ili opijatskim blokatorom posle detoksikacije

Differences in depression severity and frequency of relapses in opiate addicts treated with methadone or opiate blocker after detoxification

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Apstrakt

Uvod/Cilj. Recidiv kod opijatske zavisnosti česta je pojava nakon detoksikacije i uvođenja opijatskog zavisnika u apstinenciju od opijata. Kliničke procene ukazuju da se kod preko 90% opijatskih zavisnika ispoljavaju depresivne manifestacije u periodu posle detoksikacije, odnosno razvije se postdetoksikacijska depresija. Cilj rada bio je da se utvrde razlike u učestalosti recidiva, težini i toku depresije u periodu od šest meseci nakon detoksikacije, kao i u ranijim obrascima upotrebe opijata kod dve grupe opijatskih zavisnika, lečenih metadonom ili opijatskim blokatorom. **Metode.** Izvršeno je upoređivanje rezultata dve grupe opijatskih zavisnika: ispitanici na supstitucionoj terapiji metadonom (M) i ispitanici na terapiji opijatskim blokatorom naltreksonom (B). Kod svih ispitanika potvrđen je depresivni sindrom kliničkom i instrumentalnom procenom. Opijatski recidivi dijagnostikovani su putem panel testova za brzu detekciju metabolita opijata u urinu. Potom je ispitana povezanost recidiva sa skorovima depresivnosti i adikcionim varijablama. Primenjeni instrumenti za merenje nivoa depresivnosti bili su Hamiltonova skala depresivnosti (HAMD) i Zungova skala depresivnosti. Svi ispitanici popunili su upitnik Pompidou. Psihološka merenja vršena su tokom šestomesečnog praćenja u tri navrata. Prisustvo opijatskih metabolita u urinu kontrolisano je na svake dve nedelje. **Rezultati.** Obe grupe ispitanika (M i B) imale su visoke skorove depresivnosti na HAMD skali tokom perioda praćenja. Grupa M imala je izraženiju depresivnost tokom sva tri merenja. Uočen je pad nivoa depresivnosti u obe grupe ispitanika u funkciji vremena, koji je pratio i pad učestalosti recidiva. U obe grupe ispitanika učestalost recidiva bila je u pozitivnoj korelaciji sa ranijim adikcionim varijablama – intravenskoj aplikaciji opijata, iskustvu predoziranja, odsustvu imunizacije protiv hepatitisa C, kao i nosilaštva virusa hepatitisa C. **Zaključak.** Opijatsko recidivantno ponašanje udruženo je sa izraženom depresivnošću u postdetoksikacijskom periodu. Ispitanici grupe M imali su izraženiju depresivnost. Kod ispitanika obe grupe učestalost recidiva bila je u pozitivnoj korelaciji sa pojedinim adikcionim varijablama koje se dovode u vezu sa latentnim suicidnim ponašanjem. Dijagnostikovanje i praćenje depresivnosti opijatskih zavisnika, kao i blagovremena sanacija simptoma postdetoksikacijske depresije, prevenirale bi opijatski recidiv.

Ključne reči:
zavisnost od supstanci, poremećaji; metadon; naltrekson; depresija; lečenje lekovima; recidiv; faktori rizika.

Abstract

Background/Aim. Relapse of opiate dependence is a common occurrence after detoxification and introduction of opiate addicts in abstinence from opiates. Clinical evaluation showed that over 90% of opiate addicts exhibit depressive manifestations during detoxification, or develop post-detoxification depression. The aim of this study was to determine differences in the frequency of relapses, severity and course of depression during a of 6-month period, and previous patterns of use of opioids in the two groups of opiate addicts treated by two different therapeutic modalities. **Methods.** The results of the two groups of opiate addicts were compared: the patients on substitution methadone treatment (M) and the patients treated with opiate blocker naltrexone (B). In all the patients, clinical and instrumental evaluations confirmed depressive syndrome. Opioid relapses were diagnosed by the panel test for rapid detection of metabolites of opiates in urine. Then they were brought in connection with scores of depression and addiction variables. The Hamilton Depression Scale (HAMD) and Zunge Depression Scale were the applied instruments for measuring the level of depression. All the subjects completed a questionnaire Pompidou (short version). Psychological measurements were carried out during a 6-month follow-up on three occasions. The presence of opiate metabolites in urine was controlled every two weeks. **Results**. Both groups of patients (M and B) had high scores on

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HAMD during the study. The group on methadone had a strong depression in all three measurements. There was a drop in the level of depression in both experimental groups over time, which was accompanied by a decrease in the incidence of recurrence. In both tested groups the frequency of relapses was positively correlated with earlier addiction variables – intravenous application of opioids, the experience of overdose, the absence of immunization against hepatitis C and hepatitis C virus carriers. **Conclusion.** The opioid relapse behavior is associated with a marked depression in post-detoxification period. The tested group M had a more expressed depression which is consistent with the literature data. In both tested groups the frequency of relapses was positively correlated with individual addiction variables associated with latent suicidal behavior. Diagnosing and monitoring depression of opiate addicts as well as timely remediation of post-detoxification depression symtoms, could help in prevention of opiate relapse.

Key words:
substance-related disorders; methadone; naltrexone; depression; drug therapy; recurrence; risk factors.

Uvod

Sklonost ka recidivantnom ponašanju je najveći problem kod lečenja opijatske zavisnosti. Potraga za prediktorima opijatskog recidiva, opijatsku zavisnost sve češće dovodi u vezu sa poremećajima raspoloženja [1]. Ova vrsta poremećaja pripada depresiji kod koje su afektivna i kognitivna komponenta u drugom planu i označava se terminima "*depressio sine depressione"* ili „maskirana depresija". Simptomi maskirane depresije nazivaju se depresivnim ekvivalentima [2]. Sve je prisutnije mišljenje da pojedini oblici adiktivnog ponašanja kao što su intravenska aplikacija opijata, korišćenje zajedničkog pribora za intravensko injektiranje heroina, izbegavanje imunizacije, promiskuitetno i ostalo rizično seksualno ponašanje, predstavljaju depresivne ekvivalente. Takođe, poznato je da intravenska aplikacija heroina pozitivno koreliše sa visokim skorovima na Hamiltonovoj skali depresivnosti (HAMD) [3]. Depresivne manifestacije su posebno izražene kod opijatskih zavisnika koji povremeno uspevaju da uspostave apstinenciju. Procena je da preko 90% zavisnika ispoljava depresivne smetnje udružene sa suicidnim ponašanjem po prestanku uzimanja opijata [4, 5]. Broj pokušaja i izvršenih samoubistava 20 puta je češći kod opijatskih zavisnika nego kod opšte populacije [4, 5]. Depresija major je značajnije prisutna kod opijatskih zavisnika koji su na terapiji duževremenog održavanja metadonom [5]. Klinička praksa ukazuje na pojavu depresivnih manifestacija kod preko 90% opijatskih zavisnika u postdetoksikacijskoj fazi, što se naziva postdetoksikacijskom depresijom [6]. Ova vrsta depresije je u direktnoj vezi sa upotrebom opijata i dužina njenog trajanja je do četiri meseca posle detoksikacije [7]. Javlja se kao posledica složenih mehanizama koji nastaju zbog neuroadaptacije opioidnih receptora na egzogeni opijat, kome je adikt bio izložen u dužem vremenskom periodu [8].

Cilj ovog rada bio je da se ustanovi razlika u učestalosti pojave opijatskih recidiva kao posledice postdetoksikacijske depresije, kao i pojedinih oblika rizičnog adiktivnog ponašanja koja se dovode u vezu sa depresivnim ekvivalentima i latentnim suicidnim ponašanjem kod bolesnika lečenih metadonom i bolesnika lečenih blokatorom opioidnih receptora.

Metode

Istraživanje je sprovedeno u Službi za psihijatriju Opšte bolnice Leskovac tokom 2008. i 2009. godine, kao klinička prospektivna studija u trajanju od šest meseci, sa studijom preseka u tri navrata. Studijom su bile obuhvaćene dve grupe opijatskih zavisnika: grupa lečena metadonom (M) i grupa lečena naltreksonom, opijatskim blokatorom (B).

Ispitanici obe grupe odabrani su metodom slučajnog izbora na osnovu konsekutivnog prijema u vremenskom periodu od šest meseci. Svi ispitanici bili su muškog pola, starosti od 18–55 godina, sa najmanje jednom godinom opijatske adikcije, lečenjem u kontinuitetu od 6 meseci, pri čemu su tokom perioda praćenja imali minimalno 75% negativnih nalaza na prisustvo metabolita opijata u urinu.

Ispitanici su trijažirani u dve grupe u zavisnosti od parametara adikcione anamneze (starost, broj i vrsta prethodnih terapijskih modaliteta čijom primenom nije uspostavljena trajnija apstinencija, broj *drug free* dana, motivisanost za ponuđeni terapijski modalitet...). Tokom trijažiranja bolesnika, poštovane su njihove potrebe, ali se prevashodno rukovodilo kriterijumima propisanim metadonskim vodičem [9]. Svi ispitanici su bili podeljeni u petogodišnje starosne grupe, počev od 20 godina, koliko je imao najmlađi ispitanik. Obe grupe ispitanika imale su po 30 ispitanika. Ispitanici koji su usmeravani ka eksperimentalnoj grupi B, u adikcionoj anamnezi imali su duže apstinencione periode i *drug free* dane, koji predstavljaju indikator veće uspešnosti u apstiniranju [10].

Ispitanici obe grupe testirani su primenom HAMD skale koja sadrži 17 stavki [11], i Zungove skale [12], nakon dve nedelje, tri meseca i šest meseci od početka istraživanja. Pomoću HAMD skale interpretiraju se skorovi na sledeći način: 0–7 bez depresivnosti, 8–15 minor depresija, 16 i više major depresija. Primenom Zungove skale depresivnost se kvantifikuje izračunavanjem Zungovog indeksa, pri čemu je vrednost indeksa 0,33 označava stanje bez depresivnosti, 0,74 depresivnost koja zahteva bolničko lečenje, 0,39 depresivnost u remisiji, 0,53 depresivnost kod ostalih psihijatrijskih poremećaja. Psihološke instrumente primenila su dva nezavisna ispitivača, psihijatar i psiholog, a potom su korišćene srednje vrednosti dobijenih skorova (HAMD) i koeficijenta depresivnosti (ZUNG). Recidivantno ponašanje praćeno je primenom panel testa za brzu detekciju metabolita opijata u urinu (AbuGnost MOP300cut off, Biognost, Zagreb, Croatia). Testiranje su sprovodili obučeni tehničari u strogo kontrolisanim uslovima sa ciljem postizanja verodostojnosti uzorka i dobijenih rezultata. Kontrola apstinencije sprovodila se na dve nedelje. Opijatski adikti obe eksperimenalne grupe popunili su skraćenu verziju Pompidou upitnika [8] za sociodemografske i adikcione varijable (učestalost korišćenja opijata, način upotre-

be, iskustvo predoziranja, *i.v*. aplikacija opijata u prethodnih šest meseci, korišćenje zajedničkog pribora za injektiranje, nosilaštvo virusa hepatitisa C, imunizacija protiv hepatitisa B). Ispitanici su dali pisanu saglasnost za učešće u istraživanju.

U analizi rezultata, u zavisnosti od prirode samih podataka, korišćeni su Mann Whitney-*U* test za procenu značajnosti razlike dve nezavisne grupe heterogenih podataka, $\chi 2$ za procenu značajnosti razlike učestalosti gradacije obeležja posmatranja dva nezavisna uzorka i *t*-test za ispitivanje značajnosti razlike aritmetičkih sredina dva velika nezavisna uzorka.

U svim primenjenim statističkim metodama nivo značajnosti bio je $p < 0,05$, a nivo visoke statističke značajnosti $p < 0,01$.

Analiza podataka obavljena je korišćenjem programa za statističku obradu podataka SPSS 10.0 za Windows (*Statistical Package for Social Sciences).*

Rezultati

Ispitanici na terapiji metadonom bili su sa prosečno stari 31,87 ± 9,95 godine, dok su ispitanici na terapiji opijatskim blokatorom naltreksonom bili prosečno stari 28,03 ± 4,30 godine. Najmlađi ispitanik na terapiji metadonom imao je 21, a najstariji 59 godina. Najmlađi ispitanik na terapiji opijatskim blokatorom imao je 20, a najstariji 39 godina. Primenom *t*-testa nije dobijena statistički značajna razlika u prosečnoj starosti ispitanika posmatranih grupa ($t = 0,601$; $Df = 58$, $p > 0,05$) (slika 1).

U odnosu na sve ispitanike, srednjoškolsko obrazovanje imalo je 91,7% ispitanika, osnovnu školu 6,7% ispitanika, dok je 1,7% bilo bez završene osnovne škole (tabela 1).

Prema bračnom statusu u celokupnom uzorku preovladavali su neoženjeni (78,3%), a potom razvedeni (11,7%). Samo 6 (10%) ispitanika bilo je u braku (tabela 1). Svi ispitanici bili su nezaposleni.

Tokom perioda praćenja kod ispitanika obe grupe došlo je do pojave 104 opijatska recidiva. U grupi ispitanika M pojava recidiva je bila učestalija (69) u odnosu na grupu ispitanika B (35) (tabela 2). Primenom Mann-Whitney U-testa nije dobijena statistički značajna razlika u broju recidiva kod ispitanika grupe M i grupe B ($p > 0,05$).

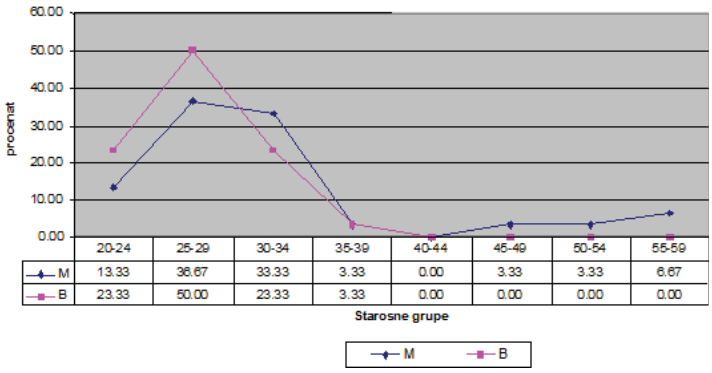
Recidivantno ponašanje je u obe grupe ispitanika bilo prisutnije na početku praćenja i imalo je trend opadanja sa napredovanjem lečenja, uz pojavu povećanja recidivantnosti u trećem mesecu praćenja kod obe grupe (slika 2). Ovaj nalaz svakako pobuđuje pažnju i zahteva praćenje na većem uzorku.

Ispitanici čija je dužina adikcije bila od 1–4 godine, tokom perioda praćenja realizovali su najveći broj recidiva. Podjednak broj recidiva ostvarili su ispitanici čija se dužina adikcije kretala od 5 do 9 i od 10 do 14 godina (tabela 2).

Primenom $\chi 2$ testa nije ustanovljena statistički značajna razlika u broju recidiva u odnosu na dužinu adikcionog staža kod obe grupe ispitanika.

U tabeli 3 prikazana je povezanost broja recidiva sa adikcionim varijablama: učestalost korišćenja opijata, način upotrebe droge, iskustvo predoziranja, intravenska upotreba opijata u poslednjih mesec dana, korišćenje zajedničkog pribora, nosilaštvo virusa hepatitisa C i imunizacija protiv hepatitisa B.

Najveći broj recidiva u obe grupe (M-69, B-26), činili su oni ispitanici koji su opijate uzimali svakodnevno, što je statistički značajna razlika u odnosu na one ispitanike koji su



Sl. 1 – Distribucija ispitanika na terapiji metadonom (M) i opijatskim blokatorom (B) prema starosnoj strukturi

Tabela 1

Nivo obrazovanja i bračni status opijatskih zavisnika na terapiji metadonom (grupa M) i opijatskim blokatorom (grupa B)

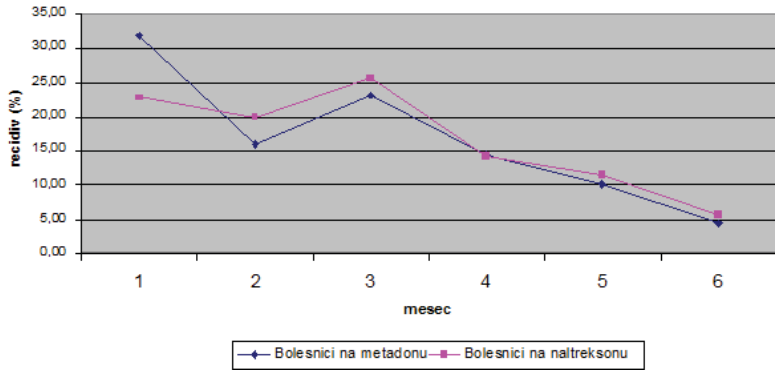
Karakteristike ispitanika	grupa M (n = 30)	grupa B (n = 30)	Ukupno (n = 60)
	n (%)	n (%)	n (%)
Nivo obrazovanja			
bez obrazovanja	1 (3,3)	0 (0,0)	1 (1,7)
osnovna škola	3 (10,0)	1 (3,3)	4 (6,7)
srednja škola	26 (86,7)	29 (96,7)	55 (91,7)
Bračni status			
u braku	3 (10,)	3 (10,0)	6 (10,0)
neoženjen	23 (76,7)	24 (80,0)	47 (78,3)
razveden	4 (13,3)	3 (10,0)	7 (11,7)

Tabela 2

Distribucija recidiva u odnosu na adikcioni staž

Adikcioni staž (god.)	Broj recidiva					
	Grupa M		Grupa B		Ukupno	
	n	%	n	%	n	%
1–4	27	39.13	19	54.29	46	44.23
5–9	19	27.54	10	28.57	29	27.88
10–14	23	33.33	6	17.14	29	27.88
Ukupno	69	100.00	35	100.00	104	100.00

Grupa M – lečeni metadonom; Grupa B – lečeni opijatskim blokatorom; $\chi^2 = 1,21$; df = 2; $p > 0,05$



Sl. 2 – Učestalost recidiva po mesecima praćenja kod ispitanika lečenih metadonom i ispitanika lečenih opijatskim blokatorom naltreksonom

Tabela 3

Distribucija recidiva u odnosu na adikcione varijable

Adikciona varijabla	Broj recidiva		Statistički parametri
	grupa M n (%)	grupa B n (%)	
Učestalost korišćenja opijata			
svakodnevno	69 (72,6)	26 (27,4)	$X^2 = 19,42$, df = 1, $p <$
nekoliko puta nedeljno	0 (0)	9 (100)	0,01
Način upotrebe opijata			
i.v. primena	66 (78,6)	18 (21,4)	$X^2 = 29,24$, df = 1, $p <$
nazalna primena	3 (15,0)	17 (85,0)	0,01
Iskustvo predoziranja			
ne	34 (52,3)	31 (47,7)	
da, jednom	12 (92,3)	1 (7,7)	$X^2 = 15,55$, df = 3, $p <$
da, više puta	20 (87,0)	3 (13,0)	0,01
ne seća se	3 (100)	0 (0)	
Da li je uzimao intravenski u proteklih mesec dana			
da	52 (86,7)	8 (13,3)	$X^2 = 26,23$, df = 1, $p <$
ne	17 (38,6)	27 (61,4)	0,01
Korišćenje zajedničkog pribora			
da	0 (0)	1 (100)	
ne	69 (67,0)	34 (33)	
Nosilaštvo virusa hepatitisa C			
test pozitivan	19 (47,5)	21 (52,5)	$X^2 = 10,34$, df = 1, $p <$
test negativan	50 (78,1)	14 (21,9)	0,01
Imunizacija protiv hepatitisa B			
da	12 (60)	8 (40)	
ne	55 (67,9)	26 (32,1)	
nepoznato	2 (66,7)	1 (33,3)	

Grupa M – lečeni metadonom; Grupa B – lečeni opijatskim blokatorom

opijate uzimali nekoliko puta nedeljno. U odnosu na adikcionu varijablu „način upotrebe droge“, intravenski korisnici opijata imali su značajno češće opijatski recidiv nego nazalni konzumenti droge. Ispitanici koji su imali iskustvo predoziranja recidivirali su u statistički značajno većem broju, u odnosu na one opijatske zavisnike koji iskustvo predoziranja

nisu imali ili ga se ne sećaju. Statistički značajnije recidivirali su i oni ispitanici koji su mesec dana pre dolaska na lečenje opijat koristili intravenski. Znatno skloniji recidiviranju bili su HCV pozitivni ispitanici. Poređenje broja recidiva po ispitaničkim grupama u odnosu na varijable „korišćenje zajedničkog pribora“ i „imunizacija protiv hepatitisa B“ nije bilo moguće zbog zanemarljivo malog broja ispitanika koji su koristili zajednički pribor, odnosno malog uzorka u odnosu na ponuđene tri mogućnosti za varijablu koja se tiče imunizacije protiv hepatitisa B.

Tokom merenja skorova depresivnosti u tri vremenska razmaka – inicijalnom, srednjem i finalnom, primenom HAMD skale, dobijene su visoke vrednosti u obe grupe ispitanika (tabela 4). Kod obe grupe uočen je i pad depresivnosti u funkciji vremena sa napredovanjem terapijskog procesa. Depresivni skor bio je najveći kod obe grupe ispitanika u inicijalnom merenju, kod grupe M čak 27,46. Skorovi depresivnosti opadali su u trećem mesecu na 25,56 (grupa M) i 16,25 (grupa B). U finalnom merenju skorovi depresivnosti obe grupe ispitanika bili su približnih vrednosti, 17,69 (grupa M) i 14,67 (grupa B) (tabela 4).

Tabela 4

Težina depresije na Hamiltonovoj skali depresivnosti (HAMD) i Zungovoj skali depresivnosti (ZUNG) kod opijatskih zavisnika na terapiji metadonom (grupa M) i opijatskim blokatorom (grupa B)

Ocenska skala	Vreme merenje	grupa M $\bar{X} \pm SD$	grupa B $\bar{X} \pm SD$
HAMD	inicijalno	27,46 ± 4,16	20,28 ± 3,13*
	srednje	25,56 ± 3,97	16,25 ± 2,64*
	finalno	17,69 ± 3,68	14,67 ± 2,15*
ZUNG	inicijalno	0,73 ± 0,04	0,51 ± 0,03†
	srednje	0,57 ± 0,03	0,39 ± 0,02†
	finalno	0,51 ± 0,02	0,37 ± 0,01†

* $p < 0,05$ vs grupa M
† $p < 0,01$ vs. grupa M

Tokom celog perioda praćenja grupa M imala je veće skorove i koeficijente depresivnosti u odnosu na grupu B.

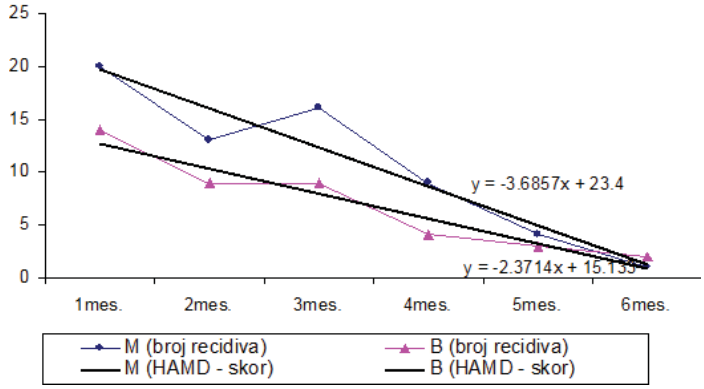
Korišćenjem SPSS paketa matematički je opisana promena nivoa depresivnosti u funkciji vremena. Kod ispitanika grupe M dobijena je relacija kojom skor depresivnosti opada sa proticanjem vremena što je predstavljeno jednačinom y = -3,6857x + 23,4. Kod ispitanika grupe B, takođe, skor depresivnosti bio je u negativnoj korelaciji sa vremenom praćenja i predstavljen je jednačinom y = -2.3714x + 15,133. Uočen je trend opadanja težine depresije i broja recidiva kod obe grupe u toku šest meseci (slika 3).

Diskusija

Tokom istraživanja ispitanici obe grupe recidivirali su 104 puta. Grupa M recidivirala je češće, ali bez statistički značajne razlike. Ovakav rezultat sugeriše ispravnu trijažu ispitanika koja je sprovedena prema smernicama metadonskog vodiča i iskustvima dobre kliničke prakse [9, 10].

Značajna statistička razlika učestalosti recidiva u odnosu na dužinu adikcije nije dobijena. Ovakav rezultat nije u saglasnosti sa podacima iz literature koji ukazuju da je uspešnost apstiniranja u negativnoj korelaciji sa dužinom adikcionog staža [13]. Razlozi za ovakav rezultat leže u kratkom vremenu praćenja ispitanika.

Nalazi koji dovode u vezu recidivantno ponašanje sa adikcionim varijablama, koje nazivamo i varijablama rizičnog ponašanja, pokazali su visoku statističku značajnost u odnosu na sledeće varijable: učestalost korišćenja opijata, način upotrebe droge, iskustvo predoziranja, intravenska upotreba u poslednjih mesec dana, nosilaštvo virusa hepatitisa C. Ove varijable dovode se u vezu sa latentnim suicidnim ponašanjem i čestom pojavom depresivnosti kod opijatskih zavisnika [14, 15]. Sve su prisutnija stanovišta da se pomenute varijable, koje su u našem slučaju bile zastupljene pre ulaska ispitanika u terapijski tretman i pre pojave



Sl. 3 – Težina depresije prema Hamiltonovoj skali depresivosti (HAMD) i broj recidiva kod obe grupe ispitanika (M, B) u toku šest meseci (M – tretman metadonom; B – tretman opijatskim blokatorom)

Primenom Zungove skale koeficijent depresivnosti bio je najveći na inicijalnom merenju u grupi M i iznosio je 0,73, dok je na srednjem merenju opao na 0,57, odnosno 0,51 na finalnom merenju. Grupa B imala je na početku merenja koeficijent depresivnosti od 0,51, koji je opao u srednjem merenju na 0,39 i 0,37 u finalnom merenju.

postdetoksikacijske depresije, zapravo depresivni ekvivalenti [1], što ukazuje na to da je depresivnost kod ispitanika postojala i pre dolaska na lečenje. Isprepletenost opijatske adikcije i depresivnosti bazira se na sličnim biohemijskim promenama koje nastaju pri primeni opijata i onima koje se javljaju kod depresije [6, 8]. Ove naučne postavke predstav-

ljaju i temelj bioloških teorija nastanka i održavanja opijatske adikcije.

Kvantifikovanjem nivoa depresivnosti u tri vremenska perioda primenom HAMD skale, dobijena je značajna statistička razlika među grupama ispitanika. Ispitanici grupe M imali su veće skorove depresivnosti u inicijalnom, srednjem i u finalnom merenju. Kod obe grupe u toku šest meseci na HAMD skali registrovana je major depresija, osim na finalnom merenju u grupi B, kada je skor od 14,67 odgovarao minor depresiji. Kvantifikovanjem nivoa depresivnosti primenom Zungove skale samoprocene, uočena je, takođe, statistički značajna razlika u nivoima depresivnosti ispitanika grupe M u sva tri vremena merenja. Ovi rezultati su u skladu sa podacima iz literature koji ukazuju da postdetoksikacijska depresija kod opijatskih zavisnika na terapiji metadonom ima klinička obeležja major depresije [16–18].

Analizirajući skorove depresivnosti primenom HAMD i Zungove skale u funkciji vremena praćenja, dijagnostikovali smo postdetoksikacijsku depresiju kod obe grupe ispitanika, pri čemu je depresivnost bila izraženija kod ispitanika grupe M. Sa odmicanjem terapijskog procesa skorovi depresivnosti su imali trend opadanja i međusobnog približavanja. Razlike u skorovima depresivnosti na početku lečenja u korist grupe M očekivane su, s obzirom na to da se radilo o ispitanicima koji su imali teži oblik opijatske adikcije i veći broj bezuspešnih lečenja primenom različitih terapijskih modaliteta, što i sam metadonski vodič nalaže kao kriterijum za usmeravanje klijenata ka supstitucionom terapijskom modalitetu. Sa napredovanjem terapijskog procesa i ulaženjem u poslednje mesece praćenja, pad skorova depresivnosti je u saglasnosti i sa samim trajanjem postdetoksikacijske depresije, koja je ograničena na period od četiri meseca [7].

Tokom našeg istraživanja zapaženo je da je tendencija opadanja depresivnosti pratila i tendenciju smanjenja broja recidiva. U raspoloživoj literaturi nismo naišli na istraživanja koja se bave pitanjem povezanosti recidivantnog ponašanja, tj. relapsa opijatomanije sa pojavom postdetoksikacijske depresije. Postdetoksikacijska depresija bila je predmet istraživanja u smislu procene suicidalnog rizika, za koji se ustanovilo da je izvesniji ukoliko je stepen depresivosti izraženiji [18]. Podaci iz literature ukazuju i na istraživanja vezana za sanaciju postdetoksikacijske depresije uz primenu antidepresiva druge generacije. Utvrđeno je da je neophodno praćenje koncentracije metadona u plazmi ispitanika koji se paralelno tretiraju antidepresivnom terapijom [19], kao i praćenje kliničke slike onih opijatskih zavisnika koji paralelno sa blokatorom opijatskih receptora, koriste i antidepresivnu terapiju [20, 21].

Utvrđivanje razlika u nivoima depresivnosti i recidivantnosti u postdetoksikacijskom periodu kod opijatskih zavisnika u dva terapijska modaliteta otvorilo je i pitanje povezanosti depresivnosti i recidivantnog ponašanja. Prepoznavanje eventualne prediktorske uloge depresivnosti u recidivantnom ponašanju zahteva opsežniji psihološki instrumentarijum, veći uzorak opijatskih zavisnika i duže vreme praćenja, što su i ograničenja naše studije. S obzirom na to da je centralno pitanje u lečenju opijatskih zavisnika, kako prevenirati recidivantno ponašanje, predlog za buduća istraživanja odnosio bi se na identifikovanje onih psihijatrijskih parametara koji bi mogli imati prediktorsku ulogu u recidivantnom ponašanju.

Zaključak

Tokom istraživanja opijatski zavisnici na terapiji metadonom češće su recidivirali, ali bez statistički značajne razlike u odnosu na grupu na terapiji opijatskim blokatorom. Recidivantno ponašanje kod obe grupe ispitanika u odnosu na adikcione varijable koje pripadaju rizičnom ponašanju, bilo je statistički značajno. S druge strane, rizično ponašanje se dovodi u blisku vezu sa latentnom suicidalnošću. Postdetoksikacijska depresija dijagnostikovana je kod obe grupe ispitanika, ali je teži klinički tok sa statistički značajnije višim skorovima na primenjenim skalama tokom celog perioda praćenja evidentiran u grupi ispitanika na terapiji metadonom. Ova grupa je sve vreme trajanja postdetoksikacijske depresije imala klinička obeležja depresije major. Tendencija opadanja depresivnosti pratila je tendenciju opadanja recidivantnosti u obe grupe ispitanika, što upućuje na povezanost postdetoksikacijske depresije i relapsa.

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Knowledge of nursing students about central venous catheters

Studenti nege i njihovo teoretsko poznavanje centralnih venskih katetera

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Abstract

Background/Aim. Central venous catheters (CVC) are at the crucial importance, particulary in the intensive therapy units. In order to handle a CVC safely, nursing students need to acquire theoretical and practical knowledge during the course of their studies. The aim of the study was to establish theoretical knowledge of nursing students about the procedures of nurses in placing and removing a central venous catheter (CVC), dressing the catheter entry point, the reasons for measuring central venous pressure (CVP), possible complications and risk factors for developing infections related to CVC. **Methods.** The questionnaire developed specifically for this cross-sectionl study was handed out to 87 full-time students and 57 part-time students. **Results.** The results show that all the surveyed nursing students know why chest radiography is carried out when inserting a catheter, have relatively good knowledge of CVC insertion points, procedures carried out in case of a suspected catheter sepsis and complications and risk factors for the development of infections related to CVC. However, the study show that the majority of students have insufficient knowledge of the procedures accompanying insertion of a catheter, signs that indicate correct functioning of CVC, frequency of flushing a catheter when it is not in use and the reasons for introducing an implanted CVC. **Conclusion.** Based on the results of the study it can be concluded that the second-year nursing students have insufficient knowledge of CVC. In order to correctly and safely handle a CVC, good theoretical knowledge and relevant practical experience are needed. The authors therefore believe that, in future, the classes should be organized in smaller groups with step-by-step demonstrations of individual procedures in handling a CVC, and the students encouraged to learn as actively as possible.

Key words:
students; nurse clinicians; knowledge; questionnaires; catheterization, central venous; infection; risk factors.

Apstrakt

Uvod/Cilj. Centralni venski kateteri (*central venous catheters* – CVCs) veoma su važni, naročito u jedinicama za intenzivnu negu. U cilju pravilnog rukovanja CVCs neophodno je da studenti nege steknu i teoretsko i praktično znanje tokom svojih studija. Cilj ove studije bio je da se kod studenata nege utvrdi nivo njihovog teoretskog poznavanja postupaka nege u postavljanju i izvlačenju CVC, previjanju ulaznog mesta katetera, razloga za merenje centralnog venskog pritiska (*central venous pressure* – CVP), mogućih komplikacija i faktora rizika od nastanka infekcija od CVCs. **Metode.** Urađen je upitnik specijalno za ovu unakrsnopoprečnu studiju i podeljen redovnim (n = 87) i vanrednim (n = 57) studentima. **Rezultati.** Dobijeni rezultati pokazuju da svi posmatrani studenti nege znaju zašto se sprovodi radiografija pluća prilikom postavljanja katetera, relativno dobro znaju o mestima ubacivanja CVC, procedurama koje se sprovode kada se sumnja u sepsu od katetera i komplikacije i faktore rizika od nastanka infekcija od CVC. Studija, međutim, pokazuje da većina studenata nedovoljno poznaje postupke ubacivanja katetera, pokazatelje pravilnog funkcionisanja CVC, učestalosti ispiranja katetera kada je on van upotrebe, kao i razloge za ubacivanje ugrađenog (implantiranog) CVC. **Zaključak.** Prema dobijenim rezultatima ove studije može se zaključiti da studenti II godine nege nedovoljno znaju o CVCs. Za pravilno i bezbedno rukovanje neophodno je dobro teoretsko znanje o CVCs i odgovarajuće praktično iskustvo. Autori, zato, veruju da bi ubuduće nastava trebalo da se organizuje u manjim grupama, uz postupno pokazivanje svakog pojedinačnog postupka rukovanja sa CVC i motivaciju studenata za aktivno učenje.

Ključne reči:
studenti; medicinski tehničari; znanje; upitnici; kateterizacija, centralna, venska; infekcija; faktori rizika.

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Introduction

Central venous catheters (CVCs) are of crucial importance in modern medical practice, particularly in intensive therapy units. They enable intake of larger quantities of highly concentrated liquids and medications that damage peripheral veins (vesicant chemotherapy, parenteral nutrition, hypertonic antibiotic solutions), giving blood and blood product transfusions, taking blood samples for testing, monitoring hemodynamic status of critically ill patients and administering liquids and medication at a patient's home [1–4].

Despite the fact that CVC enables vitally necessary venous access, its use carries the risk of local and systemic infections [5]. CVC infections represent a serious complication in treatment, which worsens prognosis, prolongs hospitalization and increases treatment costs [6]. The highest percentage of primary blood infections are related to CVC [7]. Sepsis due to spreading of microorganisms from colonized CVCs develops in 0.9% to 8.0% of cases [8–9]. The occurrence of infection is influenced by the general condition of a patient, duration of hospitalization, anatomic catheter insertion point, number of lumens, inappropriate aseptic technique when handling CVC, type of dressing material, colonization of a catheter entry point and colonization of an attachment part of a catheter [3]. The main infection routes are extraluminal and endoluminal. Contamination risk is lowered by the use of maximal sterile barriers and the use of appropriate disinfectant [3, 10].

In a patient with an inserted CVC, the nurse carries out the aseptic technique for monitoring a catheter entry point, dressing a catheter, flushing a catheter, taking smears of a catheter entry point, adjusting the prescribed infusion liquids, parenteral nutrition, transfusion of blood and blood products, taking blood samples, measuring the central venous pressure (CVP) and application of medications. In order to handle a CVC safely, nursing students need to acquire theoretical as well as practical knowledge during the course of their studies. At the Faculty of Health Sciences, students of nursing acquire theoretical and practical knowledge in the first year of the bachelor studies in nursing. In the past, students of nursing at the Faculty of Health Sciences gained clinical knowledge and skills at clinical training in the clinical environment, due to considering this the best method for acquiring knowledge and important practical experience. Clinical situations probably do represent the best method for acquiring practical skills, however, clinical environment often does not provide optimal learning opportunities due to overcrowding and a lack of experienced clinical mentors [11]. Students often claim that they learn most when performing something on their own, which means learning through experience and solving certain problem situations [12–13].

Clinical environment and clinical situations can be successfully simulated in nursing care labs. In nursing labs, learning is active, and safe, without risks for patients safety; specific learning situations are created, possible errors of students are established and corrected and creativity of a student is encouraged. A student receives feedback from the teachers, colleagues and a "simulated" patient [14]. Successful learning in simulated situations requires clinically experienced mentors who continuously monitor changes in practice, as the scientific bases for clinical practice necessitate an understanding of biomedical science [15].

So far, no studies covering knowledge of nursing students about CVC have been published in Slovenia. Due to the importance of knowledge and the aseptic technique when handling a CVC, we were interested in how much theoretical knowledge students acquire in the first year of bachelor studies in nursing. The aim of the study was to estimate theoretical knowledge of nursing students about CVC.

Methods

This was a quantitative, single cross-section questionnaire survey of two nursing student cohorts from the Faculty of Health Science in Ljubljana, Slovenia. Eighty seven full-time and 57 part-time students of the second year of bachelor studies in nursing at the Faculty of Health Sciences (Ljubljanja, Slovenia) participated in the study. Eighty seven questionnaires were handed out to the regular students and all 87 were returned. Sixty five questionnaires were handed out to the part-time students, who returned 57 questionnaires (a return rate of 87.7%). The survey was carried out between November and December 2009. The survey was voluntary and anonymous.

The questionnaire was developed specifically for this study and included question about knowledge of procedures when inserting and dressing a catheter and about complications and risk factors for the development of infections related to CVC. The survey questionnaire included 23 questions, divided into three sections: demographic data, knowledge of procedures when inserting and dressing a CVC and knowledge of complications and risk factors for the development of infections related to CVC. The questionnaire featured closed-type and open-type questions. One question was dichotomous. A pilot study was not carried out.

The basic descriptive statistics and the *t*-test for establishing differences in average values of theoretical knowledge about CVC among the full-time and part-time students were carried out. The statistical programme SPSS v. 17 was used for data analysis.

Results

The sample included 60.4% full-time and 39.6% part-time students. Among the full-time students, 11.5% were male and 88.5% female, the age ranged from 19 to 31 years ($\overline{X}$ = 20.79; SD ± 1.61). The majority of full time students (63.2%) completed the secondary school of nursing, 17.2% completed a general upper secondary school and 19.5% other secondary schools. Among the part-time students, 24.6% were male and 75.4% female, the age ranged from 20 to 53 years ($\overline{X}$ = 29.65; SD ± 7.41). The majority (77.2%) of part-time students completed the secondary school of nursing, 8.8% completed general upper secondary school and 14% other secondary schools.

The participants' theoretical knowledge is shown in Table 1. Only 1.4% knew the veins into which CVC is most commonly inserted, 69.4% knew them partially. From 87 par-

Table 1

Descriptive statistics and differences in knowledge of Central venous catheter (CVC) between the full-time (n = 87) and part-time students (n = 57)

Questions on CVC	Number of participants (n)	Knows		Knows partially		Does not know		No answer		$\overline{x}$	SD	*t*-test	
		n	%	n	%	n	%	n	%			F	*p*
CVC insertion points	87	0		63	72.4	21	24.1	3	3.4	2.32	0.60	1.052	0.956
	57	2	3.5	37	64.9	16	28.1	2	3.5	2.31	0.54		
CVC tip positioning	87	25	28.7	0	0	42	48.3	20	23.0	1.86	0.69	0.000	0.491
	57	18	31.6	0	0	29	50.9	10	17.5	1.94	0.72		
Radiograpy following CVC insertion	87	87	100	0	0	0	0	0	0	/	/	/	/
	57	57	100	0	0	0	0	0	0	/	/		
Trendelenburg position of a patient prior to CVC insertion	87	9	6.3	0	0	37	42.5	41	47.1	3.49	0.50	6.293	.057
	57	0	0	0	0	29	50.9	28	49.1	3.26	0.91		
Valsalva manoeuvre aim	87	0	0	31	35.6	43	49.4	13	14.9	2.47	0.66	0.077	**.006**
	57	0	0	35	61.4	17	29.8	5	8.8	2.79	0.68		
Dangers of air entering the vein	87	50	57.5	0	0	31	35.6	6	6.9	1.72	0.45	6.373	0.224
	57	36	63.2	0	0	14	24.6	7	12.3	1.62	0.49		
Insertion of CVC via a tunnel aim	87	30	34.5	3	3.4	8	9.2	46	52.9	2.77	1.31	0.833	0.887
	57	15	26.3	11	19.3	3	5.3	28	49.1	2.80	1.39		
Signs of correct CVC functioning	87	8	9.2	23	26.4	48	55.2	8	9.2	2.35	0.74	0.002	**0.025**
	57	7	12.3	25	43.9	23	40.4	2	3.5	2.64	0.78		
CVC flushing frequency	87	4	4.6	0	0	78	89.7	5	5.7	1.13	0.43	18.132	**0.032**
	57	3	5.3	0	0	52	91.2	2	3.5	1.34	0.72		
Use of 10 ml syringe for flushing CVC	87	49	56.3	0	0	34	39.1	4	4.6	2.11	1.13	1.325	0.326
	57	28	49.1	0	0	24	42.1	5	8.8	1.92	1.07		
Frequency of replacing infusion system	87	39	44.8	0	0	43	49.4	5	5.7	2.31	0.51	1.592	0.287
	57	34	59.6	0	0	18	31.6	5	8.8	2.60	2.37		
Stoppers with no-return valves frequency replacing	87	24	27.6	0	0	58	66.7	5	5.7	1.83	1.55	0.106	0.339
	57	25	43.9	0	0	28	49.2	4	7.0	1.61	0.75		
Transfusion	87	48	55.2	0	0	34	39.0	5	5.7	2.51	0.70	0.882	0.521
	57	32	56.1	0	0	19	33.4	6	10.5	2.43	0.75		
Reason for stopping application of medications through CVC	87	9	10.3	0	0	75	86.2	3	3.4	2.61	0.86	11.350	0.113
	57	12	21.1	0	0	43	75.4	2	3.5	2.83	0.65		
Purpose of measuring CVP	87	31	35.6	0	0	31	35.6	25	28.7	1.60	0.78	0.152	**0.014**
	57	33	57.9	0	0	14	24.6	10	17.5	1.93	0.80		
Use of sterile gloves in CVC dressing	87	54	62.1	0	0	33	37.9	0	0	1.07	0.26	136.489	**0.000**
	57	51	89.5	0	0	4	7.0	2	3.5	1.38	0.49		
CVC dressing frequency	87	32	36.8	0	0	54	62.1	1	1.1	3.09	4.28	4.186	0.314
	57	17	29.8	0	0	40	70.2	0	0	2.43	2.94		
Disinfecting entry point in CVC dressing	87	58	66.7	0	0	28	32.2	1	1.1	2.74	0.52	2.772	0.286
	57	44	77.2	0	0	13	21.8	0	0	2.64	0.55		
Complications in patients with inserted CVC	87	4	4.6	83	95.4	0	0	0	0	5.67	2.34	0.219	0.059
	57	0	0	57	100.0	0	0	0	0	6.43	2.33		
Risk factors for CVC infections	87	2	2.3	81	93.1	0	0	4	4.6	2.18	0.57	3.646	0.239
	57	0	0	52	91.2	0	0	5	5.8	2.07	0.45		
The most common paths for CVC infections (extraluminal)	87	38	43.7	0	0	42	48.3	7	8.0	2.64	4.56	0.863	0.563
	57	28	49.1	0	0	22	38.7	7	12.3	3.61	13.85		
Procedures with patients in case of suspected catheter sepsis	87	4	4.6	78	89.7	0	0	5	5.7	1.93	0.37	2.462	0.177
	57	6	10.5	49	86.0	0	0	2	3.5	2.01	0.32		

ticipants 63.2% of the full-time and 84.2% of the part-time students knew that a CVC is inserted into the subclavian vein; 42.5% of the full-time and 49.1% part-time students knew that it is inserted into the jugular vein and 23% of the full-time and 40.4% part-time students knew that it is inserted into the femoral vein; 28.7% of the full-time and 31.6% part-time students knew that, as a rule, the tip of an inserted CVC lies in the superior vena cava; 48.3% of full-time and 50.9% part-time students did not know this fact. All the surveyed students knew that following insertion of CVC into the subclavian vein or the superior vena cava, a radiography of the lungs and the heart is carried out in order to check the position of a catheter. Prior to CVC insertion, a patient is placed into the Trendelenburg position with the aim to prevent air from entering blood stream and the occurrence of pulmonary embolism; only 6.3% of the full-time and none of the part-time students knew this. None of the participants knew the purpose of the Valsalva maneuver

when inserting and removing CVC, 35.6% of the full-time and 61.4% part-time students gave a partially correct answer. A total of 14.9% of the full-time and 19.3% part-time students of the participants knew that the Valsalva breathing technique causes a rise in intrathoracic pressure; 20.7% of the full-time and 31.6% of the part-time students knew that it diminishes blood flow to the heart and 29.9% of the full-time and 45.6% part-time students knew that the Valsalva maneuver is performed in order to prevent air from entering blood stream and to prevent air embolism. The danger of air entering CVC is higher when CVP is lower; a correct answer was given by 57.5% of full-time and 63.2% part-time students; 34.5% of the full-time and 26.3% part-time students knew why a doctor decides to insert CVC through a tunnel under the skin.

Only 9.2% of the full-time and 12.3% part-time students knew all the signs indicating correct functioning of CVC; 26.4% of the full-time and 43.9% part-time students provided a partially correct answer. A total of 66.7% of the full-time and 84.2% part-time students knew that when CVC functions correctly, needle aspiration results in blood in a syringe; 54% of the full-time and 59.6% part-time students knew that in this case, infusion runs well; 43.7% of the full-time and 57.9% part-time students knew that blood returns into the infusion system when bottle is lowered below the level of a patient's heart, and 54% of the full-time and 40.4% part-time students knew that a patient does not report discomfort or have signs of other complications. Only 4.6% of the full-time and 5.3% part-time students correctly answered the question on how often CVC needs to be flushed when not in use. A total of 56.3% of the full-time and 49.1% part-time students knew that a 10 ml syringe is used to flush CVC, as these results in lower pressure in the lumen of a catheter and consequently lowers the possibility of catheter damage. A total of 44.8% of full-time and 59.6% part-time students knew that the frequency of replacing infusion systems, stopcocks and splitters depends on the type of liquid infused *via* CVC; 27.6% of the full-time and 43.9% part-time students knew the stoppers with non-return valves for syringe handling need to be replaced every 72 hours.

A total of 55.2% of full-time and 56.1% part-time students knew that transfusions of blood and blood products are applied and blood samples taken *via* CVC only if other options are not available; 10.3% of full-time and 21.1% part-time students knew that when resistance is felt to applying medication *via* CVC, application must be stopped immediately and a doctor notified. A total of 35.6% full-time and 57.9% part-time students knew the purpose of measuring CVP; 35.6% full-time and 24.6% part-time students gave a wrong answer, while 62.1% full-time and 89.5% part-time students knew that sterile gloves must be used when dressing CVC. A total of 36.8% full-time and 29.8% part-time students knew that dressing of CVC entry point should be carried out as needed. A total of 66.7% full-time and 77.2% part-time students knew that when dressing CVC, entry point should be disinfected three times.

Only 4.6% full-time students knew all the listed most common complications with CVC; 95.4% full-time and all part-time students knew them partially. Infections were listed by 95.4% full-time and 94.7% part-time students, clogging of a catheter by 79.3% of full-time and 71.9% part-time students, catheter falling out by 73.6% full-time and 66.7% part-time students, wrong direction of a catheter 70.1% of full-time and 45.6% part-time students, hematoma by 55.2% full-time and 66.7% part-time students, catheter sepsis by 57.5% full-time and 61.4% part-time students and air embolism by 63.2% full-time and 45.6% part-time students.

Only 2.3% full-time students knew all the listed risk factors for the development of infection, related to CVC; 93.1% full-time and 91.2% part-time students knew risk factors partially; 90.8% full-time and 84.2% part-time students listed incorrect cleaning of CVC entry point as a risk factor for the development of infection; 73.6% full-time and 78.9% part-time students listed failure to use the aseptic technique and maximum sterile barriers when handling CVC, 77% full-time and 66.7% part-time students listed incorrect handling with CVC and 20.7% full-time and 17.5% part-time students listed frequent use of CVC. The most common infection route for CVC is the extraluminal route; 43.7% full-time and 45.8% part-time students gave a correct answer.

Only 4.6% full-time and 10.5% part-time students knew all the listed procedures carried out in case of a suspected catheter sepsis; 89.7% of full-time and 86% part-time students knew the procedures partially. A total of 65.5% of full-time and 59.6% part-time students knew that in case of a suspected catheter sepsis, two hemocultures are taken (one through CVC and the other from the patient's peripheral vein); 54% full-time and 77.2% part-time students knew that a catheter tip needs to be sent for microbiological tests; 32.2% of full-time and 24.6% part-time students knew that a smear of CVC entry point is taken in case signs of inflammation are present.

The *t*-test for independent samples showed a statistically significant difference between the full-time and part-time nursing care students in the following aspects of knowledge CVC (see Table 1). Part-time students had important knowledge of the purpose of the Valsalva maneuver ($F = 0.077$, $p = 0.006$; 95% CI 0.545–0.094), signs of correct functioning of CVC ($F = 0.002$, $p = 0.025$; 95% CI 0.545–0.037), frequency of flushing the CVC ($F = 18.132$, $p = 0.032$; 95% CI 0.600–0.069), purpose of measuring the CVP ($F = 0.152$, $p = 0.014$; 95% CI 0.431–0.181) and importance of wearing sterile gloves when dressing a CVC ($F = 136.489$, $p = 0.000$; 95% CI 0.401–0.018).

Discussion

The study shows that the majority of full-time and part-time second-year nursing students completed the secondary nursing school. Such an educational structure was expected, as the study programme of nursing in Slovenia is mostly chosen by candidates who have completed the secondary nursing school. The result analysis showed that part-time students more frequently gave correct answers to some questions.

In general, the students' knowledge about CVC is relatively insufficient. Only 3.5% part-time students know all the typical CVC inserting spots, the majority of participants only partially know them. Some of the participants do not even possess knowledge of all the Latin names for veins used for inserting CVC, which indicates insufficient knowledge of anatomy and physiology.

Following CVC insertion, the tip of a catheter lies in the superior vena cava. Only 28.7% of full-time and 31.6% part-time students gave this answer. The recommended position of the tip is in the lower third of the superior *vena cava*, immediately preceding the entry into the right atrium [16]. When inserting CVC into the femoral vein, the tip of a catheter lies in the inferior *vena cava* [2].

The Trendelenburg position is known as the position of the body with the head downwards, which is achieved by lowering the head of the bed by 10 to 20 degrees [17] or 10º–30º [18]. The patient is placed in deep Trendelenburg position prior to insertion and during removal of CVC in order to prevent air embolism [19]. Only a small percentage of full-time students knew that a patient should be placed in the Trendelenburg position in order to prevent air from entering blood stream and air embolisms. None of the part-time students knew the answer to this question, which was very surprising.

The Valsalva maneuver causes pressure in the thorax to rise and blood flow to the heart to diminish, which lowers the possibility of air entering through an open needle. A couple of seconds following CVC removing, the Trendelenburg position and the Valsalva maneuver prevent air from entering the space of a catheter placement and enable closing of the vein [19]. A good third of the participants only partially knew the effects of the Valsalva maneuver. None of the students listed all the three correct claims, which indicated a poor knowledge of physiology. The danger of air entering blood stream and development of pulmonary embolism is higher, if CVP is lower; 57.5% full-time and 63.2% part-time students gave a correct answer. Part-time students had a higher number of correct answers. Radiography control of catheter position is needed for all central venous catheters supplying liquids into the subclavian vein or the superior *vena cava* [20]. All the participants knew that radiographic imaging is used to control the position of a catheter, which is commendable.

Only one third of the participants knew that the doctor decides on insertion of a tunnelled central venous catheter when the patient requires long-term and intensive treatment. It is true that full-time nursing students are rarely faced with implanted catheters in clinical practice; however, within the framework of lab classes, the students are given enough information on this type of catheter.

Prior to each use of CVC for applying medications or infusion liquids, it is necessary to check the passage through and correct functioning of a catheter. Signs indicating a partial or total occlusion of catheter need to be considered seriously and a full passage through a catheter established [21]. Approximately 10% of the participants stated that correct functioning of CVC may be deduced from the following facts: when aspiration results in blood in a syringe, when infusion is running, when blood returns to the infusion system after a bottle has bee lowered below the level of a patient's heart and when a patient does not report any discomfort or has signs of other complications. A good third of the participants only partially knew these signs. Flushing CVC is necessary to ensure flow through a catheter [22]. It prevents formation of fibrin linings, even though fibrin linings occur in all CVCs to a certain degree [2]. Depending on the type of catheter 0.9% NaCl and heparin solution are used for flushing. Commonly accepted flushing methods are flushing with positive pressure and the stop-start technique [4]. It is accepted that flushing CVC twice a week prevents catheter clogging. The survey results show that only 4.6% full-time and 5.3% part-time students correctly answered the question on CVC flushing frequency when it is not in use.

It is very important that not too great force is used when flushing CVC in order to prevent catheter ruptures and catheter embolism. Many instructions recommend the 10 mL syringe as the smallest admissible [4]. In our survey, a good half of the participants correctly answered the question about why a 10 mL syringe is used to flush the catheter.

With clear infusion liquids, it is necessary to replace the system and connectors every 72 hrs, except in case of disconnecting infusion catheter from CVC [10]. In general, infusion systems are replaced after expiration of the prescribed time period for system use (every 12, 24, 48 or 72 hrs). When blood and blood products or fat emulsions combined with amino acids and glucose are given through a catheter, infusion system needs to be replaced every 24 hrs [6]. When propofol infusion is given, infusion system needs to be replaced every 6 to 12 hrs [23]. In our survey, half of the participants correctly answered that the frequency of replacing infusion system and infusion connectors depends on the type of infusion liquid running through CVC.

Stoppers with non-return valves need to be replaced less frequently than infusion systems, which means every 72 hrs [2, 3]. A good third of the participants knew that stoppers with non-return valve need to be replaced every 72 hrs. As expected, the part-time students more frequently provided a correct answer. Over half of the participants knew that blood or blood product transfusion and taking of blood samples are carried out *via* a CVC only if no other option is available.

Central venous pressure is pressure inside the superior vena cava and the inferior vena cava with usually equals the pressure in the right atrium. CVP values depend on the balance between the venous inflow into the right atrium and the pumping ability of the right ventricle. CVP is a good indicator of the fluid balance in the body and the heart function, especially right-sided heart failure and pulmonary edema [3, 24]. A total of 35.6% of full-time and 57.9% part-time students knew why CVP is measured. This question was also more frequently answered correctly by the part-time students.

Many studies support the use of bandages; however, bandage type remains disputed [25]. To cover CVC entry point, transparent bandages that enable monitoring of a catheter entry point and purpose bandages that ensure CVC is carried out safely (even without sutures) are used [26]. Transparent bandages are replaced every 7 days or more frequently in

case of inflammation or wet, dirty or bloody bandages [10, 26]. In our survey, the majority of participants knew that sterile gloves are required when dressing CVC entry point, which is commendable. A total of 66.7% full-time and 77.2% part-time students know that catheter entry point should be cleaned/disinfected at least three times. A good third of the participants knew that CVC dressing should be carried out as required, which indicated insufficient knowledge.

Treatment of patients with CVC must be oriented towards lowering the risk for the development of complications and recognizing the signs of complications as soon as possible [4]. The study results show that 95.5% of full-time and all the part-time students have very good knowledge of the most common complications occurring with a CVC.

Approximately 20% of nosocomial infections develop due to the use of CVC. A 4-year study carried out in England showed that 43% of infections are related to CVC [27]. Colonization of the skin at CVC insertion spot plays an important role in the colonization of a catheter and entry of infection into blood stream [28]. The results of our survey show that the participants have good knowledge of the majority of risk factors for the development of CVC infections. Incorrect cleaning of catheter entry point and incorrect handling of CVC by the nurse were the most commonly cited risk factors. The extraluminal route was cited as the most common route of CVC infections.

In a suspected catheter sepsis, taking two hemocultures is recommended (one through a catheter and the other from a peripheral vein); with signs of inflammation, a smear of catheter entry point should be taken and catheter tip sent for semiquantitative culture according to Maki [3, 19]. Only 4.6% full-time and 10.5% part-time students knew all the procedures carried out in a suspected catheter sepsis; the majority of participants provided partially correct answers. Taking blood samples for hemoculture and sending a catheter tip for microbiological analysis.

Conclusion

Monitoring and handling CVC represent very important and responsible aspects of the nurses' work. CVC infections are a dangerous, sometimes even fatal complication in treating critically ill patients. Many complications can be prevented or at least limited by high-quality care of CVC and high-quality nursing care of a patient. The results of our study show that the surveyed nursing students have insufficient knowledge of CVC. Correct and safe handling of the CVC is not possible without good practical and theoretical knowledge. The researchers believe that in order to improve the knowledge of nursing students, it is necessary to carry out practical classes in smaller groups, with step-by-step demonstration of individual procedures in handling CVC and to encourage students to learn as actively as possible.

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Neonatal screening of hearing function by otoacustic emissions – a single center experience

Neonatalno ispitivanje slušne funkcije metodom otoakustičkih emisija – iskustvo jednog centra

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Abstract

Background/Aim. Nowadays development of techniques enables detection of hearing impairment in a very short time, immediately after birth by using otoacoustic emissions. They are low-pitched sounds produced in physiologically clear cochlea and can be recorded in cochlear outer meatus. By this method, complete data are found on a whole presynaptic auditory nervous system functioning that has mostly been affected by pathological changes making it a perfect screening test. Reliability and sensibility of this method is up to 98%. The aim of this study was to present the first results of systematic neonatal screening of hearing function by otoacoustic emissions in the Clinical Center Kragujevac (Kragujevac, Serbia). **Methods.** This prospective study of neonatal hearing screening function, initiated systematically by the 2008 at the Clinical Center Kragujevac, included full-term newborns and premature born ones, within the first 24 h after birth, using a DPOAEs interacoustics otoread-screener. Retesting was done after a month. **Results.** From January 1st, 2009 to December 1st, 2010, a total number of examined infants by this method was 1,994 out of which 1,778 were full-term and 216 were premature born. The test passing was higher in the group of full-term babies (92.5%) than in the preterm ones (55.1%). No bilateral answers were recorded in premature born children compared to the full-term ones, of whom a larger number was with missing lateral responses. The results of re-examination test in the group of full-term born and premature newborns were 83.7%, and 61%, respectively. **Conclusion**. Deliberately provoked transient otoacoustic emission is an efficient method in testing hearing function in newborns, since it is non-invasive, rapid and objective. Its correlation with audibly evoked potentials is very high, which confirms its reliability.

Key words:
infant, newborn; infant, premature; hearing; hearing tests; evoked potentials, auditory.

Apstrakt

Uvod/Cilj. Razvoj tehnike danas omogućio je da se metodom otoakustičkih emisija za vrlo kratko vreme dobije uvid u stanje sluha kod deteta neposredno posle rođenja. Cilj rada bio je da se prikažu prvi rezultati sistematskog neonatalnog ispitivanja sluha metodom otoakustičkih emisija u Kliničkom centru Kragujevac (Kragujevac, Srbija). **Metode.** Ova prospektivna studija ispitivanja neonatalne slušne funkcije, započeta sistematski krajem 2008. godine u Kliničkom centru Kragujevac, obuhvatila je decu rođenu na vreme i prevremeno rođenu decu i to 24 h posle rođenja. Testiranje je vršeno pomoću aparata DPOAEs Interacoustics OtoRead-Screener, kao i retestiranje nakon mesec dana kasnije. **Rezultati.** Od 01. 01. 2009. do 01. 12. 2010. ovom metodom ispitano 1 994 novorođenčadi, od čega 1 778 rođenih na vreme i 216 prevremeno rođenih. Prolaznost na testu bila je veća u grupi dece rođene na vreme i iznosila je 92,5%, a u grupi prevremeno rođene dece 55,1%. Odgovori su izostajali obostrano kod većeg broja nedonešene dece u odnosu na decu rođenu na vreme, kod koje su izostajali jednostrano. Rezultati ponovnog pregleda pokazali su u grupi na vreme rođenih prolaznost 83,7%, a 61% u grupi nedonešenih. **Zaključak.** Prolazno izazvana otoakustička emisija je efikasan metod za ispitivanje sluha novorođenčadi, pošto je neinvazivna, brza i objektivna. Njena korelacija sa čujno izazvanim potencijalima je vrlo visoka, što potvrđuje njenu pouzdanost.

Ključne reči:
novorođenče; novorođenče, prevremeno; sluh; sluh, ispitivanje; evocirani potencijali, auditorni.

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Introduction

The importance of proper hearing function was reported even in 100 years before Christ by the Greek philosopher Epictetus, "Nature gave man two ears and one tongue so that he can hear twice more than he can say", what still counts nowadays, in time of communication necessity.

Hearing impairment can occur at any age, but the most severe one appear before or immediately after birth [1]. The consequences of these damages can cause speech and intellectual development function disorders. For these reasons, even from old times it was searched for an exact method for determining hearing function immediately after birth. Since hearing impairment is not just a personal problem, but of the whole society, nowadays centralized programs for systematic research (screening) of hearing impairment immediately after birth are conducted in order to detect and successfully treat impairment before the clinical symptoms appearance [2, 3].

Statistical data reveal that in 1,000 births, one to two newborns have hearing impairment, while in the group with risk factors, this number is higher and amounts to four [4]. Individual attempts of early detection of hearing function impairment were found in the distant past. In the 1980s, for example, a compulsory screening of neonates was introduced in the United States. It has become a mandatory diagnostic method, which demands detection of congenital hearing impairment within the first year of a child's life [5]. Since 1993, mostly in the countries of Western Europe, and recently in the neighboring countries, a universal newborn hearing-screening test has been applied. In our country, by an Act on National Program on Women, Children and Youth Health Care from April 24th 2009, a compulsory early neonatal hearing impairment screening test was introduced. Thus, the study included all newborns, those with positive test results to be controlled until hearing impairment found or excluded. Most commonly used methods are otoacoustic emission (OAE) and electrophysiological auditory brainstem response (ABR tests). Reliability of methods of OAE is estimated in the range of 80% to 98%, an automated ABR (AABR) from 84% to 90% [6].

In 1978, David Kemp first proved the presence of a feedback signal after cochlea stimulation by tones and pulses, calling them "evoked acoustic emissions". Otoacoustic emissions are low-pitched sounds that originate from physiologically clear cochlea. It is assumed that otoacoustic emissions are caused by the mobility of external cochlea cells that produce a wave by their frequencies movement, where a part of that energy returns through the oval window and inner ear and is detected in the corridor. Commonly used techniques, whose clinical reliability has been approved, are the evoked OAE, transient evoked OAE (TEOAE) and distortion product OAE – DPOAE [7]. They differ in the way they are generated and recorded but enable precise and frequency specific information. DPOAEs are generated by stimulating cochlea simultaneously by two clear sounds, which produces a third tone which differs form the two entering tones by frequency and can be separated and recorded. This method examines the frequency in the range of 1000 to 8000 Hz. In TEOAE a short-time click is used as a stimulus that activates the whole cochlea. By the DAE method, damage of the sensor-cochlea can be detected, but not its degree. If the cochlea function is normal, internally generated sound is recorded. However, in case of cochlear hearing impairment, cochlea either generates response that falls below the level that is expected for a normal hearing function or does not generate any response at all. If hearing impairment is greater than 30 dB at all frequencies, no answer is recorded. For testing, a soft probe is used, containing the microphone and micro speaker, which are placed in external auditory meatus. Automatic algorithms for response detection are implemented in the apparatus [8].

The aim of the study was to present the first results of systematic neonatal hearing function ability by a OAE method in the Clinical Center Kragujevac (Kragujevac, Serbia).

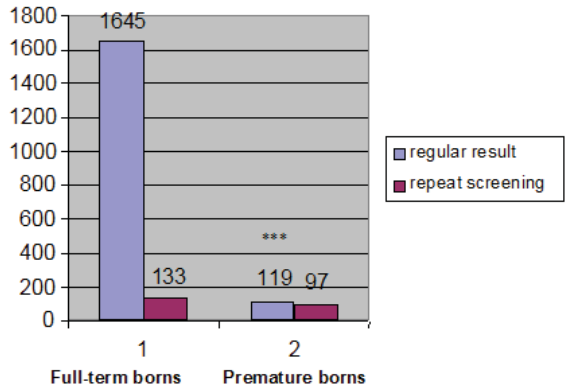
Methods

The program of systematic neonatal hearing function tests in the Clinical Center Kragujevac has been conducted since the late 2008. During this prospective study, from January 1st, 2009 until December 1st, 2010, 1,994 newborn infants were included out of who 1,778 were full-term and 216 premature born. The program was implemented according to a previously agreed protocol in collaboration with pediatricians-neonatologists. Full-term infants are examined in the Delivery Ward, within 24 h after birth – immediately after the delivery, during feeding and during sleep. Premature born children are examined in the Center for Premature Born Children when their general condition allowed that. Both ears are examined there by DPOAE Interacoustics OtoRead-screener, which is equipped with software algorithms for result recording and reading (Figure 1). Newborns with lateral or bilateral hearing impairment were scheduled for retesting in a month. The results of testing and retesting of full-term and premature born infants were then analyzed. Statistical method used was the χ^2 analysis by the Mantzel Haencel-test.

Fig. 1 – Performing neonatal screening for auditory function in the Clinical Center Kragujevac, Kragujevac, Serbia

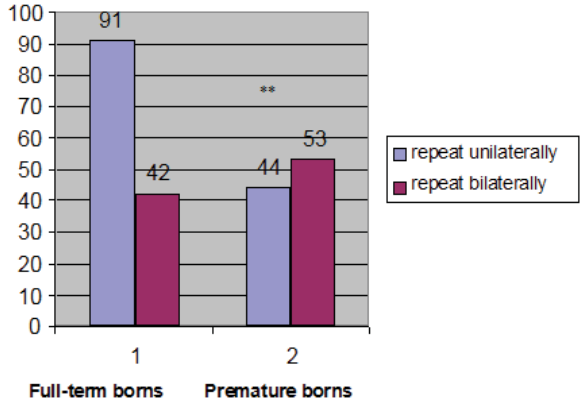
Results

Out of a total of 1,994 tested newborns, in 1,645 (92.5%) full-term infants and 119 (55.1%) premature born infants, the results were normal. Repeated examinations were required in 133 full-term born children and 97 prematurely born. The χ^2 analysis, done by the Mantzel Haencel test, showed that a significantly higher number of newborns in who repeated examinations were required was in the group of premature born children ($p < 0.001$) (Figure 2). The required repeated lateral and bilateral analysis frequency is presented in Figure 3. Conducted analysis showed that the necessity for bilateral reexamination was more frequent for the premature born children. Further testing and monitoring was necessary for 39% of preterm infants, while 83% of full-term newborns required no further monitoring (Figure 4), which is a statistically significant difference. Of the tested children, 2 full-term newborns, age 8 and 12 months, were diagnosed with a severe bilateral hearing impairment by other audiological test. They required hearing aids and auditory rehabilitation, too. One of them is a candidate for cochlear implant. Seven children required further audiological monitoring.



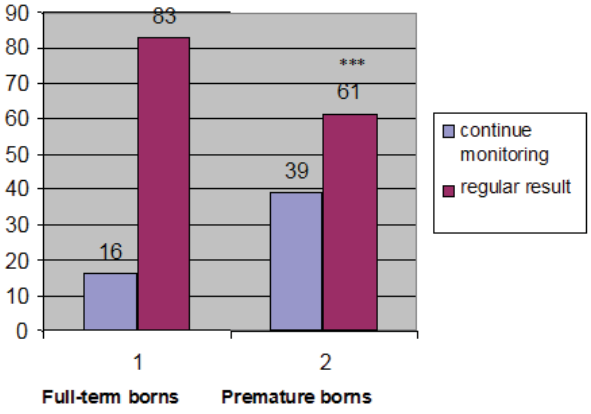
***$p < 0.001$ *vs* full-term borns children

Fig. 2 – Results of testing newborns by universal transient evoked otoacoustic emission (TEOAE) screening method



**$p < 0.01$ *vs* full-term born

Fig. 3 – The frequency of the need for unilateral or bilateral repeated screening



***$p < 0.001$ *vs* full-term born

Fig. 4 – The need for further monitoring of children following screening after a month from the first testing

Discussion

In our study, infants were at first divided in two groups – full-term delivered and premature born ones. Immediately after birth, prematurely born infants were moved to the Center for Premature Born Children, where further tests were done. In the premature born children, a number of those appeared to have positive test results on hearing impairment was recorded and the test was repeated for a large number of those infants. Out of a total number of tested infants, a higher test passing was reached in the full-term delivered children (92.5%), compared to 55.1% in preterm newborns, which represents a statistically significant difference. No response bilaterally was found in a number of premature born children compared to the full-term babies where responses were absent mostly unilaterally. After a month, retesting was done for all the children with the lack in responses, both bilaterally and unilaterally. Test passing in the group of full-term infants and in the group of preterm infants was 83.7%, and 61.2% respectively. Of the tested number, for the two full-term newborns a severe bilateral hearing impairment was detected, by other audiological tests, one at the age of 8 months and the second at the 12 months. Hearing aids were included and an auditory rehabilitation started. One of them was a candidate for cochlear implant. Seven children were scheduled for further audiological monitoring.

The results of screening by the otoacoustic emissions application in 904 newborns, at the Delivery Ward in the Clinics for Gynecology and Obstetrics at the Clinical Center "Zvezdara" in Belgrade, revealed passing on the first test in 86.3%, and in the second in 99.3% of newborns. In the two newborns unilateral hearing impairment was detected [9]. The study results of universal hearing sense screening in Sienna, Italy, in 19,000 newborns, tested by otoacoustic emission, showed that for 1.78 infant per 1,000 ones bilateral hearing impairment (35/19,700) was found. Hearing impairment di-

agnosis was set in the period up to 6 months [10]. Our research found bilateral hearing impairment in 1.05 newborn per 1,000 ones (2/1,994). The diagnosis was set in the period up to 12 months. In Australia since 2000, the universal hearing sense screening has being conducted, and so far, 25,000 newborns were tested in five main delivery wards in Perth. Results from 12,708 newborns report on screening passing of 99%, while 23/12,708 were scheduled for further auditory monitoring [11]. The screening program in newborns is considered as successful if the hearing sense unilaterally is checked for 95% of newborns. Neonatal screening of hearing function allows establishing the status of cochlea immediately after birth, because sensorineural hearing impairment in about 99% are related to abnormalities in its development [12]. The damage that is discovered and treated in the intensive establishment of synapses and the maturation of the neural auditory system from the 5th to 18th month (for children with hearing impairment hearing maturation time was extended to 4 years) has very good results, although consequences of congenital impairments can never be fully compensated [13]. Systematic testing of the hearing sense after birth required in newborn children, with special attention paid to children with prenatal or perinatal risk factors [14]. Our tests proved to be very significant. Systematic examination of hearing immediately after birth, was introduced as compulsory for all the newborn children in our town so that every newborn has to leave the maternity ward of the Center for Premature Born Children with a required importance is assigned to orderly hearing and that this is a real way to fight all the consequences of deafness. Mandatory neonatal screening of newborns for hearing impairment has not yet been implemented everywhere by our government, although there are now reliable methods available for its early detection as well as the protocol about its mandatory application. The reasons for this are inability to recognize the problem, ignorance, low health education and culture and health services oversights. Basically, this is done only in major medical centers, and when hearing impairment is suspected, a child is audiologically processed. It is easier to detect severe hearing impairment, while children with mild and moderate hearing impairments are often treated as children of lower intellectual ability or as mentally retarded children [15]. Application of universal neonatal hearing screening reduces the time of diagnosis setting and beginning of treatment in children with congenital hearing loss. Bibliography data indicate that prior to introduction of screening in the diagnosis, severe hearing impairment was diagnosed at the age of 12 to 13 months, medium hard impairment about the age of 17 months, while the introduction of screening reduced the age to 3 to 6 months [16]. The introduction of screening for hearing loss in newborns is also important from the economic aspect. The US National Center for Review, Evaluation and Management of Hearing Screening reports that detection and treatment of hearing impairment at birth for just one child saves about $ 400,000 in special education costs [17]. For introduction of neonatal screening for hearing impairment, as well as for its improvement, it is considered significant to improve data systems to support surveillance and monitoring, ensure that all children receive screening, capacity development of services, as well as to promote the importance of early detection [18].

Screening of hearing impairment program in newborns is considered successful if the hearing sense is tested unilaterally in 95% of newborns. The less number of false positive results and false negative ones for hearing impairment the better screening quality. Good screening includes less than 3% false positive results, and none with a negative false result [19]. It must be added that a certain number of children, mostly with risk factors, can develop a sensory hearing impairment, after birth and later, even after the good result on screening test. Results of screening of those neonates can be considered as false negative, however, it is important to recognize this risk category of children and retest them within the first 6 months [20]. By the screening method of otoacoustic emissions, it was impossible to detect retrocohlear hearing nerve impairment, CNS damages, functional hearing problems and central disorder of the speech message. Thus, additional research and professional monitoring is recommended for children whose risk factors (hypoxia, hydrocephalus, intracranial bleeding) can increase the possibility of this type of damage. This shows the complexity of this problem, which requires a comprehensive approach of the professional team of: audiologists, geneticians, neonatologists, defectologists, psychologists, preventive services, so that by application of a series of diagnostic, informative-educational, intervention and evaluative approaches early hearing impairments would be detected [21]. Causes of false positive results are mostly malformation and obstacles at the level of outer and medial ear, but those children must be treated with further diagnostic procedure up to a definite diagnosis. Despite the risk of false positive and false negative results, neonatal screening must be applied as compulsory in the whole country.

Conclusion

Neonatal screening for hearing function using otoacoustic emissions is a reliable and easy-to-perform test and this is exactly the right way to fight the effects of deafness.

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Correlation of subtraction parathyroid scintigraphy with weight, pathohistologic finding and oxyphil cell content of parathyroid glands in parathyroid hyperplasia

Korelacija nalaza suptrakcione scintigrafije paratiroidnih žlezda liposolubilnim radiofarmacima sa veličinom, patohistološkim nalazom i procentom oksifilnih ćelija kod bolesnika sa hiperplazijom paratiroidnih žlezda

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Abstract

Background/Aim. Parathyroid hyperplasia (PHP) is defined as an absolute increase in the mass of parenchymal cells of the parathyroid gland. PHP is classified as primary, secondary and tertiary. The enlargement of parathyroid glands (PG) is usually asymmetric, resulting in a "dominant" gland. In order to confirm the diagnosis, at least two glands should be examined histologically. Subtotal parathyroidectomy, i.e. removal of the three PG and leaving a small remnant of the forth, is the treatment of choice. High percent of PHP recurrence imposes the need for preoperative high sensitivity localizing procedures. Parathyroid scintigraphy localizes about 60% of hyperplastic glands. The aim of this study was to correlate findings of subtraction parathyroid scintigraphy (SPS) with weight, pathohistologic finding and oxyphil cell (OC) content of PG in patients with primary, secondary and tertiary parathyroid hyperplasia. **Methods.** Twenty-seven patients with primary/secondary PHP underwent SPS before surgery. Scintigraphic results were graded from 1–5, in relation to the degree of uptake. SPS graded 3, 4 and 5 were considered positive. The number and weight of operated PG were evaluated macroscopically. Pathohistologic and cellular types were defined on standard stained hematoxylin-eosin slides. OC content was defined as a percent of OC and graded from 1 to 3: grade 1 < 10%, grade 2 ≥ 10% and grade 3 ≥ 20% of OC. **Results.** SPS localized dominant gland in all patients with sensitivity 100%, and 51 from 73 hyperplastic PG, with sensitivity per gland of 70%. PG weighed 0.1 g to 6.7 g (median 1 g). A significant positive correlation ($p < 0.0001$) was found between the SPS results and PG weight. A significant positive correlation was found between PG weight and OC content ($p = 0.0002$). An insignificant correlation was found between SPS and OC content. Thirty-eight PG had < 10% of OC, 32 PG had ≥ 10% and 3 PG had ≥ 20% of OC. Four patients had diffuse PHP and 23 patients nodular PHP. There was no statistically significant difference in SPS results compared to hyperplasia type, and between OC content and hyperplasia type. A significant positive correlation ($p = 0.05$) was found between PG weight and hyperplasia type. **Conclusion.** A high positive correlation was found between SPS results and PG weight, PG weight and OC content and PG weight and hyperplasia type. Between SPS results and OC content, and between SPS results and hyperplasia type, an insignificant correlation was found. Our results showed that SPS is a reliable and very sensitive diagnostic tool in detecting abnormal PG in parathyroid hyperplasia, reaching 100% sensitivity in detecting a "dominant gland" and sensitivity per localized gland of 70%. Causes that affect increased uptake of liposolubile Tc99m radiopharmaceuticals (RF) in the hyperfunctional PG tissue and conditions which prevent RF admission into the PG cells still remain to be accurately and precisely determined.

Key words:
radionuclide imaging; sodium pertechnetate tc 99m; parathyroid diseases; parathyroid glands; diagnosis; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Paratiroidnu hiperplaziju (PHP) definišemo kao apsolutno povećanje mase parenhimskih ćelija paratiroidnih žlezda (PŽ). Paratiroidna hiperplazija može biti primarna, sekundarna ili tercijarna. Mogu biti podjednako zahvaćene sve žlezde, ali su najčešće asimetrično uvećane, uz postojanje „dominantne" žlezde. Da bi se postavila histološka dijagnoza PHP treba da budu pregledane najmanje dve PŽ. Suptotalna paratiroidektomija, koja predstavlja kompletno uklanjanje tri žlezde i ostavljanje malog dela četvrte PŽ je hirurgija izbora. Veliki procenat ponovne pojave bolesti na-

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laže potrebu za preoperativnim otkrivanjem lokalizacije svih hiperplastično izmenjenih PŽ dijagnostičkim metodama visoke osetljivosti. Scintigrafija paratiroidnih žlezda lokalizuje oko 60% hiperplastično izmenjenih PŽ. Cilj ovog rada bio je da se utvrdi povezanost pozitivnosti nalaza suptrakcione scintigrafije PŽ (SSPŽ) Tc99m-liposolubilnim radiofarmacima (RF) sa težinom, patohistološkim nalazom i procentom oksifilnih ćelija (OKĆ) u patohistološkom supstratu PŽ, kod bolesnika sa primarnom, sekundarnom i tercijarnom hiperplazijom PŽ. **Metode**. Kod 27 bolesnika sa primarnom/sekundarnom PHP preoperativno urađena je suptrakciona scintigrafija paratiroidnih žlezda. Scintigrafski nalazi ocenjivani su ocenom od 1 do 5 u zavisnosti od stepena nakupljanja RF u PŽ. Scintigrami sa ocenom 3, 4 i 5 uzimani su kao pozitivni nalazi. Makroskopskom obradom odstranjenih PŽ analiziran je broj i masa žlezda. Na stalnom preparatu, standardnim hematoksilin-eozin bojenjem, utvrđivan je patohistološki supstrat, kao i ćelijska predominacija. Procenat OKĆ ocenjivan je na sledeći način: 1 – manje od 10%, 2 – jednako ili više od 10% i 3 – jednako ili više od 20% OKĆ, na stalnom preparatu. **Rezultati.** Scintigrafija je lokalizovala domonantnu PŽ kod svih bolesnika, sa osetljivošću 100%, kao i 51 od 73 hiperplastične PŽ, sa osetljivošću po broju otkrivenih žlezda od 70%. Hiperplastične PŽ težile su od 0,1 g do 6,7 g (medijana 1 g). Značajna pozitivna korelacija nađena je između scintigrafskog nalaza i težine PŽ ($p < 0{,}0001$) i težine PŽ i procenta OKĆ ($p = 0{,}0002$). Nije nađena značajna korelacija između scintigrafskog nalaza i procenta OKĆ. Trideset osam PŽ imalo je < 10% OKĆ, 32 PŽ imalo je ≥ 10% i 3 PŽ imalo je ≥ 20% OKĆ. Četiri bolesnika imala su difuznu PHP, a 23 bolesnika nodularnu PHP. Nije bilo značajne razlike u scintigrafskom nalazu u odnosu na vrstu hiperplazije, kao ni procenta OKĆ u odnosu na tip hiperlazije. Značajna pozitivna korelacija nađena je između težine PŽ i vrste hiperplazije ($p < 0{,}05$). **Zaključak**. Visoka pozitivna korelacija nađena je između: nalaza SSPŽ i težine PŽ, težine PŽ i procenta OKĆ i težine PŽ i vrste hiperplazije. Između nalaza SSPŽ i procenta OKĆ i nalaza SSPŽ i vrste hiperplazije nije nađena značajna korelacija. Naši rezultati pokazali su da je SSPŽ pouzdana i vrlo osetljiva dijagnostička metoda u lokalizovanju hiperplastično izmenjenih PŽ, sa osetljivošću 100% u otkrivanju „dominantne žlezde" i 70% po broju otkrivenih hiperplastičnih PŽ. Dodatne uzroke koji utiču na pojačano nakupljanje Tc99m-liposolubilnih RF u hiperfunkcionalnom tkivu PŽ, kao i one koji sprečavaju ulazak i zadržavanje RF u ćelijama PŽ još uvek treba tačnije i preciznije odrediti.

Ključne reči:
scintigrafija; natrijum pertehnetat tc 99m; paratireoidne žlezde, bolesti; paratireoidne žlezde; dijagnoza; senzitivnost i specifičnost.

Introduction

Parathyroid hyperplasia (PHP) is defined as an absolute increase in mass of parenchymal cells of the parathyroid gland (PG). It is found in 7% of the PGs examined during a routine autopsy. At least two glands should be examined histologically in order to establish the diagnosis. Macroscopically PG can be of normal size or significantly enlarged. All glands can be affected equally, but enlargement is usually asymmetric, resulting in a "dominant" gland [1]. Parathyroid hyperplasia combines hyperplasia of the chief cells and oxyphil cells (OC) and it most commonly occurs secondary to hyperplasia of the chief cells. Hyperplasia can be diffuse and nodular. Parathyroid hyperplasia is classified as either primary (pPHP), secondary (sPHP) or tertiary (tPHP) correspondingly to primary (pHPT), secondary (sHPT) and tertiary hyperparathyroidism (tHPT) [2–11].

Primary parathyroid hyperplasia occurs in approximately 15% of patients with pHPT. Most cases are sporadic. Approximately 20% of pPHP is the result of chief-cell hyperplasia and is associated with some of hereditary syndromes, most importantly multiple endocrine neoplasia syndromes one and two (MEN1 and MEN2). PGs were altered in 90% of MEN1 and 30–40% of MEN2 cases [7, 9, 12–15].

Secondary PHP occurs within sHPT, most commonly as a result of renal insufficiency. Renal failure influences phosphate excretion resulting in decrease of calcium level and increased parathyroid hormone (PTH) secretion. Secondary PHP can also occur due to: vitamin D deficiency, hypomagnesaemia, malnutrition, urinary calcium excretion, influence of certain medicaments. Tertiary PHP represents an autonomous PG function in patients with previously established sHPT. The existence of tHPT is typical for patients with chronic renal failure on dialysis [6, 16].

Surgical removal of the PG is the most successful treatment of HPT. Classic procedure involving bilateral exploration and dissection of the offending PG in the neck has a 95% success rate in all patients. Failure to localize the ectopic PG and undiagnosed multiple PG disorder in pHPT represents the main reason for insufficiently successful surgical intervention. Parathyroid surgery was also indicated for patients with hipercalcemia, high PTH level and/or renal osteodystrophy in sPHP which cannot be successfully medicated. Another criterion for surgery is the detection of enlarged PG by medical imaging. Subtotal parathyroidectomy, i.e. removal of the three PG and leaving a small remnant of the fourth is the treatment of choice, even though removal of all PG with autotransplantation of the parathyroid tissue in the sternocleidomastoid muscle or the forearm is also recommended [17, 18].

Introduction of ultrasound diagnostics and subtraction parathyroid scintigraphy (SPS) *via* liposolubile radiopharmaceuticals (RF) *i.e.* Tc99m-MIBI, and Tc99m-tetrofosmin (TRF) has significantly increased the detection of the enlarged PG in the last ten years. Diagnostics facilitates surgical procedures and also decreases the recurrence and the need for reoperation [17]. The main advantage of subtraction parathyroid scintigraphy (SPS) is a high sensitivity (up to 97%) and specificity (up to 95%) in localizing solitary pHPT adenoma [19, 20]. Scintigraphy sensitivity varies in pPHP, sPHP and tPHP, rating between 15% and 60% [21]. Small adenomatous and hiperplastic PG can incite false negative findings.

False positive findings are usually caused by coincidental nodus of the thyroid gland. A large number of analyses indicates a positive correlation between scintigraphic detection and the size of hyperfunctional PG. A number of analyses suggests that the intensity of Tc99m-MIBI uptake is in positive correlation with nodular hyperplasia and OC content in PG. A correlation of positive scintigraphic findings with weight and pathohistologic finding of the hyperfunctional PG has been the subject of a number of studies [22–28]. Inability to recognize hyperplasia in pHPT and a high percentage (10–70%) of disease recurrence within sHPT and tHPT requires determination of factors which influence the increase in RF uptake intensity and PG scintigram sensitivity, particularly at hyperplastically altered glands.

The aim of this study was to correlate findings of subtraction parathyroid scintigraphy (SPS) with weight, pathohistologic finding and oxyphil cell (OC) content of the PG in patients with primary, secondary and tertiary parathyroid hyperplasia.

Methods

The research included 27 patients operated for pHPT or sHPT at an average age of 50 (27–74) years with a total number of 73 hyperplastically altered PG. All of the patients underwent SPS before surgery. Seven patients at an average age of 64.4 (52–74) years had pHPT and 20 patients at an average age of 44.7 (20–73 years) had sHPT. Eighteen patients underwent SPS with Tc99m-MIBI, while 9 patients underwent SPS with Tc99m-tetrofosmin. Static scintigrams of the head, neck and chest were performed 15 min after *iv* injection of 740 MBq of Tc99m-MIBI/TRF. Anterior projection images were obtained using an ADAC gamma camera with a low-energy, high-resolution collimator in zoom mode in 128 × 128 matrix size with a 20% energy window, connected. 2 000 000 impulses per position were gathered. Later scintigrams of the head, neck and chest were performed 2 h and 3 h after *iv* injection of Tc99m-MIBI/TRF. Four to 24 h *iv* injection of Tc99m-MIBI/TRF after washout of RF from the PG and the thyroid gland, *iv* injection of 185 MBq Tc99m-pertechnetat (deposited exclusively in thyroid gland) was administered. Ten minutes after *iv* injection of Tc99m-pertechnetat, static scintigrams of head and neck in the anterior projection were performed, gathering 2 000 000 impulses per position. After normalization of early (Tc99m-MIBI/TRF) scintigram and (Tc99m) scintigram and motion correction, subtraction Tc99-petrechnetat from Tc99m-MIBI/Tc99m-tetrofosmin scintigram was performed. Zones of the increased uptake of Tc99m-MIBI/TRF visible in subtraction image represent the hyperfunctional tissue of the enlarged and altered PG. Scintigraphic (SPS) findings were graded 1–5: grade 1 – normal RF uptake, grade 2 – discretely increased RF uptake, grade 3 – slightly increased RF uptake, SPS grade 4 – increased RF uptake, 5 – clearly increased RF uptake. SPS grades 1 and 2 were considered negative findings (−). SPS results grade 3 were evaluated as suspicious findings (±), while grades 4 and 5 were considered positive findings (+).

Number, weight and mass of the removed PGs were analyzed macroscopically, while intraoperative, *ex tempore* analysis was done microscopically on standard hematoxylin-eosin stained slides in order to determine the substrate: adenoma, type of hyperplasia, distribution and substitution of the main, light, OC and degenerative alterations. OC percentage was graded: 1 – for < 10%, grade 2 – for ≤ 10%, and grade 3 – for ≥ 20% of OC by a standard preparation method.

SPS sensitivity was calculated by the equation used to calculate matrix sensitivity. A correlation between SPS findings and PG weight; SPS and OC percentage; PG weight and OC percentage was determined using the Spearman's rank correlation test. Differences in the pathohistologic findings (diffuse or nodular hyperplasia) in relation to: scintigraphic finding, PG weight and OC number, were determined using the rank sum test (Mann-Whitney). A significance level was $p < 0.05$.

Results

Positive SPS results were recorded in all of the patients, i.e. at least a single enlarged PG was localized (scintigraphy sensitivity per patient was 100%). SPS localized 48 out of 73 hyperplastic PG (SPS grade 5–39 PG, SPS grade 4–9 PG), 22 glands were not visible (SPS grade 2–4 PG, SPS grade 1–18 PG), while findings in 3 PG were evaluated as suspicious, SPS grade 3. SPS sensitivity per localized gland, along with 3 suspicious findings, was 70% (Figure 1–3). The PG in patients with positive and suspicious scintigraphy results weighed from 0.1 g to 6.7 g (median 1g). There was no statistically significant difference in the weight of the hyperplastic PG in relation to the type of hyperparathyroidism. PG weight in patients with pHPT went from 0.2 g to 2 g (median 0.75 g), in patients with sHPT it went from 0.1 g to 6.7 g (median 0.8 g).

A significant positive correlation ($p < 0.0001$) between SPS results and weight of the hyperplastic PG was found. Glands invisible in scintigraphic scanning weighted between 0.1 g and 1 g (median 0.5 g). In patients with positive and suspicious SPS results, PG weighted from 0.1 g to 6.7 g (median 1 g). Glands invisible in scintigraphic scanning (SPS grades 1 and 2) were significantly smaller than PG localized by scintigraphy (SPS grades 3, 4 and 5) ($p < 0.001$) (Figure 4).

A significant positive correlation between PG weight and OC percentage ($p < 0.001$) was also noted. Thirty-eight PG had < 10% of OC, thirty-two PG had ≤ 10% of OC and three PG had ≥ 20% of OC in them. A notable percentage of OC was found in hyperplastically altered PG of larger weight (Figure 5).

There was no significant correlation between SPS results and percentage of OC in PG. Positive SPS results noted 23 PG with < 10% of OC, 24 PG ≥ 10% of OC and 3 PG ≥ 20% of OC. False negative SPS results (significantly smaller PG) noted 15 PG with < 10% of OC, 8 PG ≤ 10% of OC. Even though there was no statistically significant positive correlation found between SPS results in relation to OC percentage, 65% of small weight PG with SPS grades 1 and 2 (negative findings) had < 10% of OC.

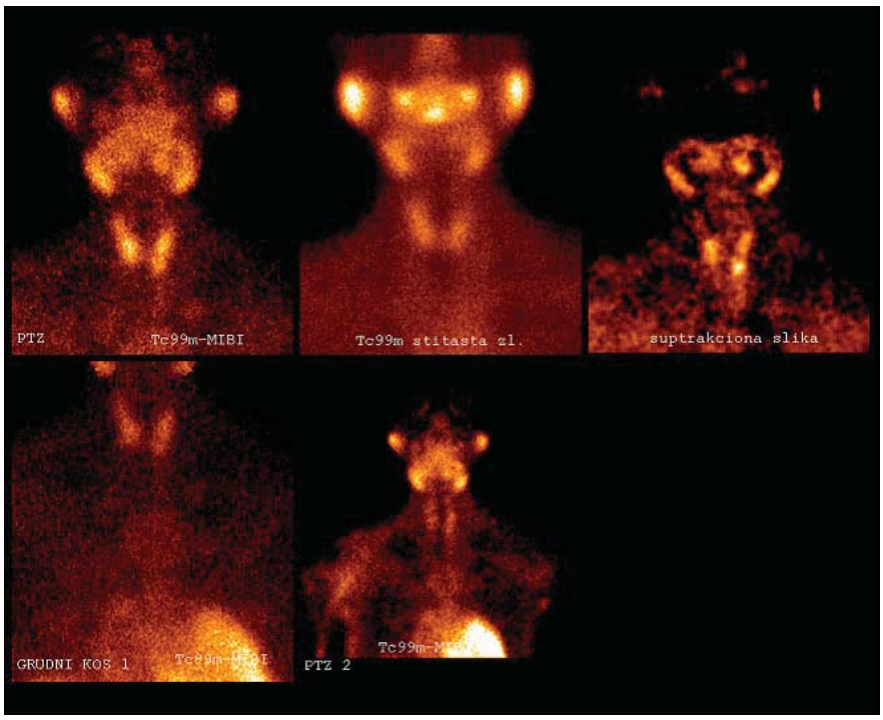

Fig. 1 – A 20-year-old, patient with secondary hyperparathyroidism (sHPT) on peritoneal dialysis 2 years and 2 years on hemodialysis, parathiroid hormone (PTH) – 1683 pg/ml, three scintigraphically positive parathyroid glands (PG): subtraction parathyroid scintigraphy (SPS) grade 4 – 0.2 g, SPS grade 4 – 0.3 g, SPS grade 5 – 0.3 g, histopathologic findings – diffuse hyperplasia, > 10% of oxyphil cells in all PG.

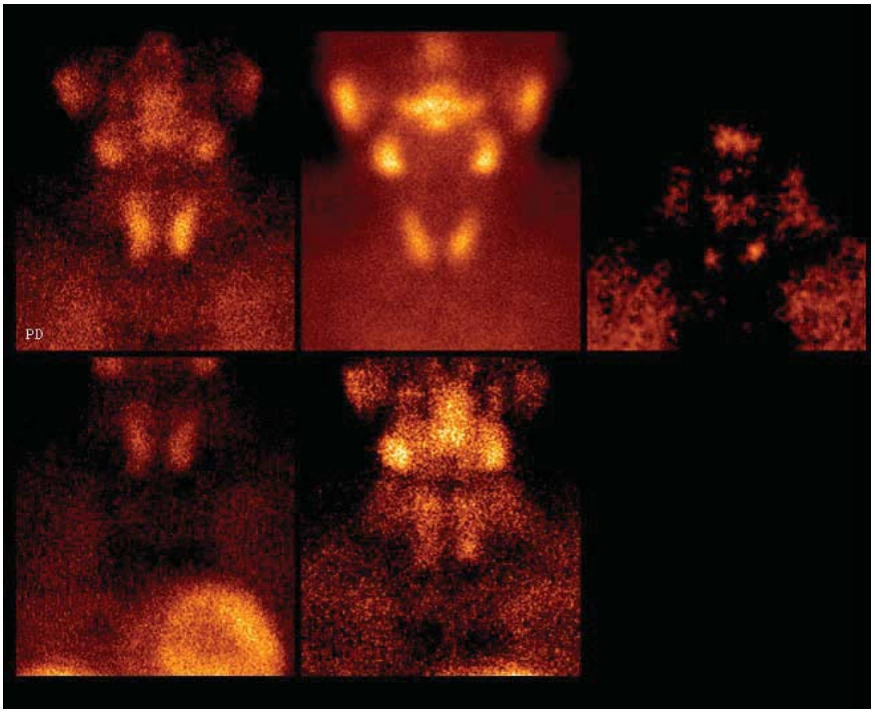

Fig. 2 – A 41-year-old patient with secondary hyperparathyroidism (sHPT) on peritoneal dialysis for 7.5 years, parathiroid hormone (PTH) – 828 pg/mL, positive scintigraphic finding in 3 of 4 parathyroid glands (PG): subtraction parathyroid scintigraphy (SPS) grade 5 – 0.8 g; SPS grade 1 – 0.3 g; SPS grade 5 – 1 g; SPS grade 5 – 1.5 g, histopathologic finding – nodular hyperplasia, oxyphil cells percent in PG: > 10%, < 10%, < 10%, < 10% respectively.

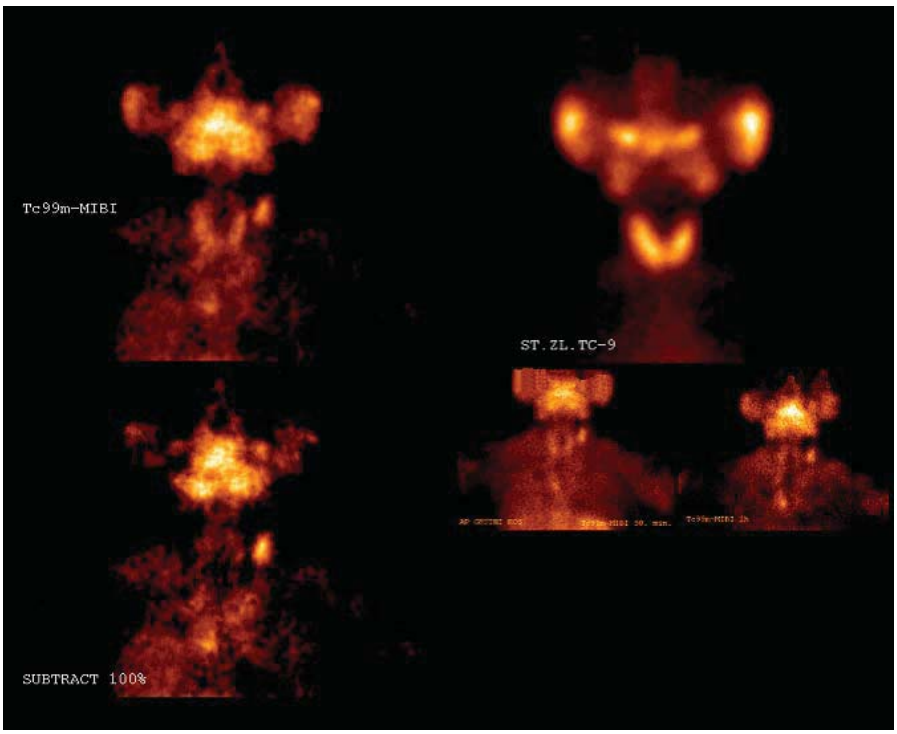

Fig. 3 – The ectopic parathyroid gland (PG) in the mediastinum of 4.5 g and the enlarged transplanted PG in the left sternocleidomastoid muscle, subtraction parathyroid scintigraphy (SPS) grade 5, in a 26-year-old patient, reccurent secondary hyperparathyroidism (sHPT), on hemodialysis 13 years, parathiroid hormone (PTH) 1500 pg/mL, histopathological finding – nodular hyperplasia, 20% of oxyphil cells.

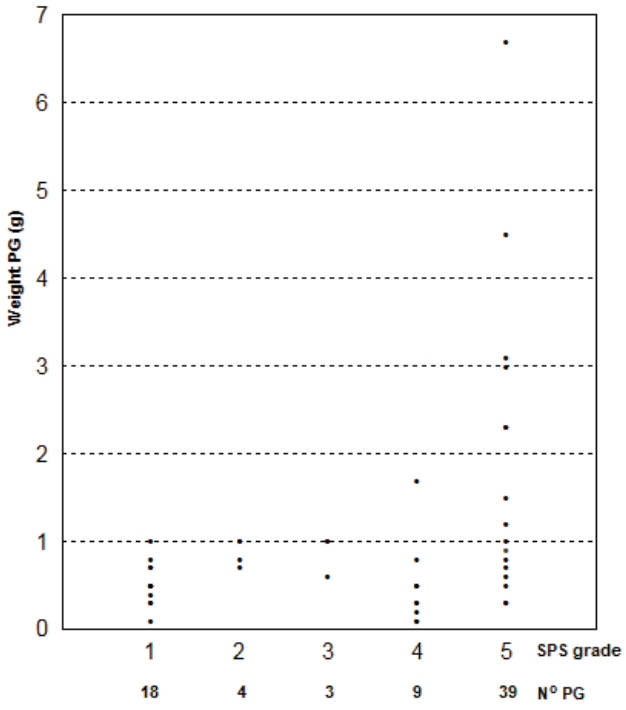

Fig. 4 – Graphical display of scintigraphic assessment findings in relation to the weight of the operated parathiroid glands (PG); x-axis: number of PG with the appropriate grade of scintigraphic findings (subtraction parathyroid scintigraphy – SPS), y-axis: weight of PG in grams.

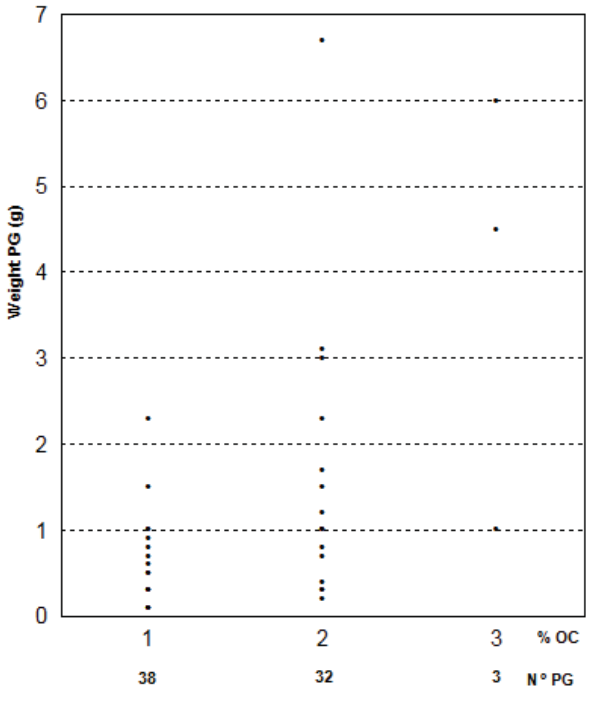

Fig. 5 – Graphical display of parathiroid glands (PG) weight in relation to the percent of oxyphil cells (% OC) of the operated parathiroid glands; x-axis: number of PG with the appropriate grade of % OC, y-axis: weight of PG in grams.

Four patients had diffuse PHP (2 patients with pHPT and 2 with sHPT) and 23 patients nodular HPH (5 patients with pHPT and 18 with sHPT). There was no statistically significant difference in SPS results in relation to hyperplasia type.

There was no significant difference between OC percentage and hyperplasia type.

A significant difference in PG weight and hyperplasia type ($p < 0.05$) was determined. The weight of the PG with diffuse hyperplasia was significantly smaller than the weight of PG with nodular hyperplasia.

Discussion

Tc99m-MIBI and Tc-99m-tetrofosmin were primarily introduced as RFs used for myocardial perfusion scintigraphy. Uptake of the two RFs is recorded in certain malignant tumors, in the PG as well as in the functional thyroidal tissue. Tc99m-MIBI and Tc-99m-tetrofosmin are intracellular markers [17, 19, 20, 28–31]. Their liposolubility enables them to travel through cellular membrane and to enter the cell. Mitochondria are responsible for RF cellular uptake, but a complete mechanism of their binding and release (perfusion, metabolic activity and phases of the cell cycle) are still relatively unknown [24, 29]. Elimination of RFs from the normal thyroid tissue is usually much faster than elimination from the hyperfunctional PG which allows PG visualisation on delayed, *i.e.* late, scintigrams, one or more hours after *iv* injection of RFs. Subtraction scintigraphy allows visualisation of glands with high RF uptake and high release rate, visualisation of intrathyroidal PG, and it also allows us to differentiate thyroid gland nodules from the enlarged PG. The hyperplastic PG can be of normal size or significantly enlarged. All of the glands can be equally affected, but the enlargement is most commonly asymmetric, resulting in a "dominant" gland. Smaller size of hyperplastically altered glands in relation to adenomatously altered PG is the main reason for the lower sensitivity of scintigraphic detection of hyperplasia [21, 22, 25, 27, 28, 32]. In our study, SPS sensitivity in localization of the largest gland was 100%, while the sensitivity in determining all of the hyperplastically altered glands was 70%. Palestro et al. [32] assume that the lower sensitivity in sPHP was caused by smaller amount of OC and the number of mitochondria which results in lower uptake and faster washout of RF from the cells. A number of analyses resulted in positive correlation between SPS and size, i.e. weight, of the hyperfunctional PG, regardless of the type of disease (adenoma, hyperplasia or carcinoma) [21, 22, 24–28, 32–34]. In our patients, we established a positive correlation between SPS results and size of the hyperplastically altered glands, both in pPHP and sPHP.

A large number of analyses determined that the intensity of Tc99m-MIBI uptake is in positive correlation with nodular hyperplasia [24, 26–28, 32]. Muros et al. [21] proved no correlation between secondary hyperplasia and SPS results. Correspondingly, there was no significant positive correlation between type of hyperplasia and positive SPS results in our patients. The results could be partly explained by a small number of PG with diffuse hyperplasia (13 patients, 6 with pHPT and 7 with sHPT), as well as by a significantly smaller PG weight.

In late 1960s, Christie [36] stated that OC of the normal PG have no secretory function and that they represent the chief cells in gland involution. A number of papers proved active secretory function of OC, both in normal and hyperfuntcional PG [4, 33, 35, 36]. OC cytoplasm is composed of tightly packed mitochondria and glycogen [16, 36]. A number of authors confirmed a positive correlation of SPS with OC content in PG [22, 24, 25, 32–34]. Yamaguchi et al. [37], as well as Bhatnagar et al [28]; Piñero et al. [38] and Ugur et al. [39] found no positive correlation between the number of OC and positive SPS results. Our group of patients also proved no positive correlation between positive SPS results and OC content in hyperplastic PG.

Apart from the factors which influence uptake kinetics of liposoluble Tc99m RF in hyperplastically altered PG, a number of other factors that might influence kinetics are being tested. This is primarily applied to factors which prevent admission and uptake of RF in PG cells. Individual histological characteristic and metabolic activity of the hyperfuntctional parathyroid tissue influence the positivity of Tc99m-MIBI and Tc99m-tetrofosmin SPS results [9, 26, 34, 38]. Furthermore, unrecognized hyperfuntcional PG were conditioned by predominance of light chief cells [22, 24–26, 40]. The obtained results are still questionable.

RF kinetics can be altered by different serum calcium levels due to its influence on the membrane potential [23, 34, 41] and mitochondrial content in PG cells [41]. It has been proven that cellular expression of P-glycoprotein (P-gp) and multiple drug resistance protein (MDR) can be a significant factor in obtaining false negative SPS results in HPT [28, 37, 42]. Concentration of MIBI and tetrofosmin in altered PG cell is in negative correlation with P-gp [28, 37, 42]. Positive correlation between the intensity of Tc99m-MIBI uptake and phases of cell cycle will be the subject of future analyses. High level of RF uptake as indicator of metabolic status is in positive correlation with the phases of positive growth of hyperfunctional parathyroid tissue [43]. Complete mechanisms of uptake and washout of Tc99m-liposolubile RF in PG are still missing and are the subject of many studies.

Conclusion

In this study the PG with diffuse hyperplasia was of significantly smaller weight than the PG with nodular hyperplasia. A significant positive correlation between SPS grade and the weight of hyperplastic PG was found. A positive correlation was also established between PG weight and OC content, but there was no statistically significant difference between SPS results depending on OC content and type of hyperplasia. Our results showed that SPS is a reliable and very sensitive diagnostic tool in detecting the abnormal PG in parathyroid hyperplasia, reaching 100% sensitivity in detecting a "dominant gland" and overall sensitivity of 70%. Causes that affect increased uptake of liposolubile Tc99m radiopharmaceuticals in the hyperfunctional PG tissue and conditions which prevent RF admission into the PG cells still remain to be accurately and precisely determined.

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Advantages and limitations of clopidogrel response testing methods

Prednosti i ograničenja metoda za testiranje odgovora na klopidogrel

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Ključne reči:
antiagregaciona sredstva; trombociti, funkcijski testovi; lekovi, toksičnost; rizik, procena; lečenje, ishod.

Introduction

Clopidogrel is a thienopyridine that irreversibly inhibits platelet P2Y12 receptors and adenosine diphosphate (ADP) mediated platelet aggregation. It is a prodrug that requires activation in the liver by cytochrome P450 enzymes (CYP3A4, CYP3A5, CYP1A2, CYP2C9, CYP2C19, and/or CYP2B6) [1].

Dual antiplatelet therapy with clopidogrel and aspirin has become the mainstay of treatment of patients with acute coronary syndromes undergoing percutaneous coronary intervention [2–4].

Clopidogrel response

Despite significant benefits of the combined antiplatelet treatment in large clinical trials, the occurrence of adverse ischemic events remains a serious clinical problem [5–7]. Clinical trials have shown that 8%–10% of patients experience a recurrent cardiovascular event during the first year after acute coronary syndromes and 1%–3% an acute or subacute stent thrombosis after percutaneous coronary intervention [8, 9].

A possible reason for these adverse events might be the fact that clopidogrel's antiplatelet effect is not uniform in all patients. Many studies have shown that individual response variability to this thienopyridine derivate and the prevalence of individuals, who are deemed to have an inadequate response to clopidogrel therapy, varies between 4% and 30% [10–12]. Unfortunately, clopidogrel resistance itself is not yet clearly understood and there is no apparent consensus on the definition of clopidogrel resistance. Also, there are significant differences between the platelet function tests used, agonist concentrations, and cut-off points. Nevertheless, the term clopidogrel resistance should generally be limited to those who fail to achieve a desired pharmacological response to drug therapy, rather than patients who experience recurrent ischemic events while on antithrombotic therapy [13].

Monitoring of clopidogrel action

Until the early 1990's bleeding time was still considered as the most useful test for the detection of platelet function. Recently, many better tests have become available that may be used to assess the influence of antithrombotic drugs on platelet. Despite that, methodological variability within each technique makes it difficult to compare results, and it is associated with unclear role of platelet function testing in clinical practice.

In this paper we discuss about the most used tests in practice which examine platelet function and agreggation, which is indirect way to evaluate response to clopidogrel.

Light transmission aggregometry (LTA) has been regarded as the gold standard for assessing platelet function for more than two decades. Aggregation of platelets is traditionally measured in platelet rich plasma (PRP) using an optical aggregometer. Aggregation response is simulated by adding of an agonist (ADP for clopidogrel). Transmission amplifies when an agonist is added, platelets aggregate and light transmission increases. Results are presented in percentage, between 0 and 100, according to the degree of light transmission [14, 15]. Also, the rate of aggregation is measured.

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LTA test is still widely used, but there are many disadvantages of that method including sample preparation. Methodology for preparation of PRP vary between different laboratories including various types of anticoagulants used (citrate or hirudin), different centrifugation speeds and times reported. Some authors argue that whole blood tests of platelet function are more reliable than PRP assays. In the latter case, isolated platelets are analyzed, which is not their physiological milieu. Also, PRP usually do not include all the platelets; the most active and larger platelets may be lost during centrifugation. In addition, other blood cells are present in the whole blood – erythrocytes and leukocytes, which also interfere with platelet aggregation. This technique is not standardized yet and different concentrations of ADP (5–20 μM) are used. LTA test takes too long, it is laboratory-based, blood samples should be sent as soon as possible to a laboratory, and it requires trained technicians. Also, LTA assay is not suitable to test on large number of samples, which is not convenient for routine clinical practice (Table 1) [15–17].

ceptors. When platelets are activated, microbead aggregation is more rapid and reproducible; therefore, platelet activation is induced when the reagent ADP/prostaglandin E_1 (ADP/PGE1) is incorporated into the assay channel. The reagent is formulated to specifically measure $P2Y_{12}$-mediated platelet aggregation. Light transmittance increases as activated platelets bind and aggregate fibrinogen-coated beads. The instrument measures this change in optical signal and expresses results in $P2Y_{12}$ Reaction Units (PRU) and a higher PRU reflects greater ADP mediated platelet reactivity. ADP is used to activate platelets by binding to the $P2Y_{12}$ and $P2Y_1$ receptors, while PGE_1 is used to reduce the ADP-induced $P2Y_1$ activation which is contributed with increase of the assay sensitivity. This assay is the most commonly used method for monitoring of clopidogrel response in USA.

The Veritas study on 147 patients has shown that VerifyNow $P2Y_{12}$ Assay is a fast and sensitive test for monitoring platelet inhibition during clopidogrel therapy [18]. The main limitations of the test are well-known: it is a closed

Table 1

Available methods for monitoring of clopidogrel response (modified from [14–16])

Method	Advantages	Disadvantages
Light transmission aggregometry	• Gold standard • Predictive of outcomes	• Not standardized test (different reagents used, different reagents concentration, different instruments) • Sample preparation • Platelet Rich Plasma test • Time consuming (1h–3h)
VerifyNow $P2Y_{12}$ Assay	• Point of-care (POC) assay • Whole blood test • Simple, fast, small sample volume • Three test cartridges • Widely used in USA • Predictive of outcomes	• Rigidity • Cartridge can only be used for single analysis • Closed system • One canal
Flow cytometry	• Whole blood test • Small blood volumes • Preparation methods flexible	• Specialized laboratories • Expensive instrument • Complex for routine monitoring Experienced operator
Multiple electrode aggregometry	• Point of-care (POC) assay • Whole blood test • Simple, fast, easy to learn • Five channels for parallel tests • Open system • Predictive of outcomes	• Relative new method • Lack of the corresponding number of studies • Comparison with other methods uncertain • No ready-made reagents

The VerifyNow System is a turbidimetric based optical detection system, which measures platelet-induced aggregation. The VerifyNow $P2Y_{12}$ assay is a whole blood, fast and standardized point of care analyzer used to measure the level of platelet $P2Y_{12}$ receptor blockade. The system consists of an instrument, a disposable assay device and quality control materials. The assay device contains a lyophilized preparation of human fibrinogen-coated beads, platelet activators, and buffer [14]. A patient`s sample is anticoagulated whole blood, which is automatically dispensed from a blood collection tube into an assay device by the instrument. The assay is based upon the ability of activated platelets to bind fibrinogen. Agglutination takes place once the activated platelets are exposed to the fibrinogen coated micro particles and in proportion to the number of the available platelet receptors. The system without the possibility of assay modification; cartridge can only be used for single analysis; delays in testing or difficulties regarding specimen collection may produce spurious results [19].

Since clopidogrel irreversibly inhibits ADP binding to the platelet $P2Y^{12}$ receptor and prevents subsequent phosphorilation of vasodilator-stimulated phosphoprotein (VASP), the increase in VASP phosphorilation could be a useful marker of clopidogrel resistance [17]. Standardized *flow cytometric* VASP *assay* is used for determination the VASP phosphorylation state of whole blood [20]. Flow citometry is a powerful technique that simultaneously measures and then analyzes multiple physical characteristics of single particles, as they flow in a fluid stream through a beam of light. Parameters analyzed with such an assay include particle's rela-

tive size, relative granularity or internal complexity, and relative fluorescence intensity. These characteristics are determined using an optical-to-electronic coupling system that records how the cell or particle scatters incident laser light and emits fluorescence. The flow cytometric assay, like Verify Now System, uses combination of ADP and PGE[1] for analysis of the clopidogrel response. Blood samples were collected in 0.129 M sodium citrate vacutainer tubes and incubated with PGE1 alone or PGE[1] and ADP, before fixation with paraformaldehyde. After this procedure, platelets were permeabilized with non–ionic detergent and labeled with a monoclonal antibody 16C2, specifically directed against serine 239 phosphorylated VASP, followed by a staining reagent, polyclonal anti-mouse antibody IgG-FITC (fluorescein isothiocyanate). Platelet population was identified on its forward and side scatter distribution and 10,000 platelet events were gated. ADP receptor reactivity was calculated using mean fluorescence intensities (MFI) in the presence of PGE1 or PGE1+ADP according the corresponding formula. There is an inverse correlation between clopidogrel treatment efficacy and the ADP receptor reactivity ratio.

The main disadvantage of flow cytometry assay is that it needs suspension of single cells or other particles, with minimum clumps and debris. This means that tissue architecture and any information about the spatial relationship among different cells are lost when single cells or nuclei are prepared. Also, this test is too complex for routine monitoring of clopidogrel and requires experienced person who will perform analysis. Other limitations are total cost of the procedure, and interactions of drugs with VASP assay (e.g. drugs affecting intracellular cyclic adenosine monophosphate and/or nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) signals may influence VASP phosphorylation) [21].

Multiple electrode aggregometry (MEA) is a new technique for detection of platelet function. This method is performed to analyze platelet function in whole blood based on impedance aggregometry. In Europe, the multiplate assay is slowly but surely winning over other methods for monitoring of clopidogrel resistance. The system registers the electrical impedance change due to the aggregation of platelets on two independent electrode set surfaces in the test cuvette and the analysis are measured simultaneously on two sensor units-internal QC. The obtained impedance is transformed to arbitrary aggregation units (AU) that are plotted against time (AUmin) [22]. Like VerifyNow P2Y12 and flow cytometry assay, MEA uses ADP and PGE1 as agonists. Main advantages of such a test involve the use of heparin and hirudin, because these anticoagulants do not interfere with serum calcium, an important second messenger of platelet activation and aggregation. Also, MEA is rapid test; smaller samples of blood are needed; up to five parallel samples could be analyzed at the same time; electronic pipetting reduces operator errors and it is easy to learn. However, MEA has not been sufficiently tested in clinical settings as a relatively new method.

In general, there is a correlation between light transmission aggregometry ("gold standard") and other tests of platelet function, e.g. VerifyNow $P2Y_{12}$ assay, VASP assay and MEA [23–26]. In addition, Varenhorst et al. [27] reported that the VerifyNow$P2Y_{12}$ correlated strongly with inhibition of $P2Y_{12}$ receptors, as measured with either VASP or LTA. The only exception is the Platelet Function Analyzer PFA-100, a reliable test for monitoring of aspirin but not clopidogrel response. Gremmel et al. [28] investigated correlation between LTA and other tests in the same time. These authors evaluated clopidogrel response in 80 patients on combined antiplatelet therapy after coronary stent implantation – throw LTA, VerifyNow$P2Y_{12}$, VASP assay, MEA and Impact R measure of platelet inhibition. The results showed that all of these methods correlated significantly with LTA, where VerifyNow$P2Y_{12}$ had the strongest correlation. Despite significant correlation between LTA and VerifyNow$P2Y_{12}$, VASP assay, MEA and Impact R, registrated sensitivities and specificities ranged from 55% to 35% and from 85% to 78.3%, respectively [28]. Unfortunately, it was not emphasized in which patients this disagreement was detected. Similarly, Paniccia et al. [29] showed that MEA significantly correlated with VerifyNow$P2Y_{12}$ with moderate agreement and 81.5% of concordant values. Also, a significant correlation was shown between MEA and LTA with good agreement and 88.8% of concordant values.

When Bland-Altman analysis was used instead of correlation, only a low agreement was found between light transmission aggregometry, whole-blood aggregometry, PFA-100 and VerifyNow $P2Y_{12}$ Assay (randomized, double-blind trial on 116 patients with stable coronary artery disease treated with clopidogrel) [30].

The importance of evaluating the (non)adequate response to clopidogrel could be seen in a possible relation with adverse cardiovascular events. Numerous studies have found that low response to clopidogrel is associated with an increased risk of ischemic events after percutaneous coronary intervention (PCI) (Table 2) [22, 31–34]. In Excelsior study, which investigated platelet function in 802 patients, throw LTA, was shown that attenuated response to clopidogrel is

Table 2

Clinical implication of inadequate response to clopidogrel [22, 31–34]

Study	Number of patients	Method	Results	Outcome
Hochholzer et al, 2006 [31]	802	LTA	↑ Platelet aggregation	30-day MACE
Buonamici et al, 2007 [32]	804	LTA	↑ Platelet aggregation	Stent thrombosis
Patti et al, (ARMYDA PRO Study)2008 [33]	160	VerifyNow $P2Y_{12}$	↑ PRU (4^{th} quartile)	30-day MACE
Sibbing et al, 2009 [22]	1608	MEA	↑ Platelet reactivity	Stent thrombosis
Siller-Matula et al, 2010 [34]	416	MEA	↑ Platelet reactivity	Stent thrombosis

MACE – major adverse cardiac events; ARMYDA-PRO – Antiplatelet Therapy for Reduction of Myocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome; PRU – platelet ($P2Y_{12}$) reaction units; LTA – light transmission aggregometry; MEA – multiple electrode aggregometry

an independent predictor of major adverse cardiac events (MACE) [31]. Also, by using LTA assay, Buonamici et al. [32] found that nonresponsiveness to clopidogrel is a strong and independent predictor of stent thrombosis in patients receiving sirolimus- or paclitaxel-eluting stents. Other studies using other methods have also reported on correlation regarding testing response to clopidogrel and cardiovascular outcomes. In ARMYDA- PRO trial platelet reactivity was evaluated in 160 patients before PCI and at 8 h and 24 h after intervention with the VerifyNow P2Y12 assay. The results have shown that pre-PCI PRU levels in the fourth quartile were associated with 6-fold increase in risk of 30-day MACE [33]. Sibbing et al. [22] used MEA to detect the response to clopidogrel. The authors found that a low response to this thienopyridine is to a significant level associated with higher risk of stent thrombosis. The predictive value of MEA was also pointed on by Siller-Matula et al. [34] who have examined platelet reactivity in their prospective study with 416 patients by the use of MEA and VASP assay. These results have shown that MEA can predict stent thrombosis better, than the VASP assay.

However, a prospective cohort study called Popular involved 1,069 patients subjected to intracoronary stent implantation and treated with clopidogrel. The primary endpoint (composite of all-cause death, nonfatal acute myocardial infarction, stent thrombosis, and ischemic stroke) occurred more frequently in patient with high on-treatment platelet reactivity detected by the LTA, VerifyNow P2Y12, and Plateletworks assay (11.7%, 13.3%, and 12.6%). Despite a significant association between those tests and the primary endpoint, their predictive accuracy was only modest [35].

We found no significant difference in one-year mortality between good and bed responders to clopidogrel in our open, prospective, controlled study on 52 participants (VASP assay) [12]. Also, myocardial infarction and/or revascularization did not occur in good or bad responders during a follow-up period of one year. However, an insufficient number of participants and open design preclude firm conclusion from those data.

Finally, the role of a reduced platelet response to clopidogrel in patients with certain comorbidities (e.g. type 2 diabetes) as compared with platelet function tests still remains to be clarified [36].

Conclusion

Thienopyridine (clopidogrel) resistance still remains to be clarified. More well-designed clinical trials with a sufficient number of participants are needed in order to draw valid conclusions. The very first step in clopidogrel resistance problem solving is to establish a unique platelet aggregation test which is reliable, effective, simple and low cost. Also, it would be useful to establish cut-off values for high on-treatment platelet reactivity that provides accurate prognostic information for high-risk patients subjected to intracoronary stent implantation.

The greatest benefit from the determination of response to clopidogrel should have patients who need a long-term usage of this drug. Based on the response to clopidogrel is possible to decide on further course of treatment, in other words, patients with inadequate response to the drug can receive either a higher dose of clopidogrel or switch to another antiplatelet drug.

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Autoimunski poliglandularni sindrom tipa 2 udružen sa mijastenijom gravis

Autoimmune polyglandular syndrome, type 2 associated with myasthenia gravis

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Apstrakt

Uvod. Udružena pojava autoimunske nadbubrežne insuficijencije sa autoimunskom bolešću štitaste žlezde i/ili prisustvom autoimunskog dijabetes melitusa tipa 1 definiše autoimunski poliglandularni sindrom tipa 2, kome se veoma retko pridružuje i mijastenija. **Prikaz bolesnika**. Prikazana je bolesnica sa autoimunskim poliglandularnim sindromom tipa 2 udruženog sa mijastenijom gravis. Kod bolesnice, stare 49 god., sa znacima mišićne slabosti prethodno je dijagnostikovana primarna nadbubrežna insuficijencija uz očuvanu morfologiju nadbubrega i niske vrednosti serumskog kortizola i aldosterona. Primarni hipotireoidizam potvrđen je niskim vrednostima slobodnog tiroksina 4 – *free thyroxine 4* (FT_4) i povišenom vrednosti tireostimulišućeg hormona (TSH), a imunska geneza dokazana je nalazom antitela na tireoidnu peroksidazu i TSH receptore. Mijastenija gravis utvrđena je prisustvom povišenog titra antitela na acetilholinske receptore, uz tipičnu kliničku sliku i dijagnostičke testove. Imunska priroda nadbubrežne insuficijencije nije se mogla potvrditi iz tehničkih razloga nivoom antitela na 21-hidroksilazu nadbubrega. Indirektan dokaz predstavljao je normalan morfološki nalaz nadbubrega, odsustvo drugih oboljenja koja mogu da dovedu do insuficijencije nadbubrega, kao i niske serumske vrednosti i kortizola i aldosterona. Po uvođenju adekvatne supstitucije hormonskim preparatima i antiholinergične terapije, uz kortikosteroidnu terapiju mijastenije gravis, kod bolesnice je došlo do poboljšanja opšteg stanja i nestanka mišićne slabosti. **Zaključak**. Prikaz bolesnice ukazuje na potrebu za pažljivim ispitivanjem bolesnika sa autoimunskim oboljenjem, zbog mogućnosti njegove udruženosti sa drugim autoimunskim oboljenjima.

Ključne reči:
poliendokrinopatije, autoimunske; miastenija gravis; komorbiditet; dijagnoza, diferencijalna; lečenje lekovima.

Abstract

Introduction. Autoimmune polyglandular syndrome type 2 is defined as adrenal insufficiency associated with autoimmune primary hypothyroidism and/or with autoimmune type 1 diabetes mellitus, but very rare vith myasthenia gravis. **Case report.** We presented a case of an autoimmune polyglandular syndrome, type 2 associated with myasthenia gravis. A 49-year-old female with symptoms of muscle weakness and low serum levels of cortisol and aldosterone was already diagnosed with primary adrenal insufficiency. Primary hypothyroidism was identified with low values of free thyroxine 4 (FT_4) and raised values of thyroidstumulating hormone (TSH). The immune system as a cause of hypothyroidism was confirmed by the presence of thyroid antibodies to peroxidase and TSH receptors. Myasthenia gravis was diagnosed on the basis of a typical clinical feature, positive diagnostic tests and an increased titre of antibodies against the acetylcholine receptors. It was not possible to confirm the immune nature of adrenal insufficiency by the presence of antibodies to 21-hydroxylase. The normal morphological finding of the adrenal glands was an indirect confirmation of the condition as well as the absence of other diseases that might have led to adrenal insufficiency and low levels of both serum cortisol and aldosterone. Hormone replacement therapy, anticholinergic therapy and corticosteroid therapy for myasthenia gravis improved the patient's general state of health and muscle weakness. **Conclusion.** This case report indicates a need to examine each patient with an autoimmune disease carefully as this condition may be associated with another autoimmune diseases.

Key words:
polyendocrinopathies, autoimmune; myasthenia gravis; comorbidity; diagnosis, differential; drug therapy.

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Uvod

Udružena pojava autoimunske nadbubrežne insuficijencije sa autoimunskom bolešću štitaste žlezde i/ili prisutvom autoimunskog dijabetes melitusa tipa 1 definiše autoimunski poliglandularni sindrom tipa 2 (APS 2). Ovaj tip poliglandularnog sindroma češće se javlja od ostalih autoimunskih poliglandularnih sindroma, a smatra se da je prevalencija 1,4–2,0 na 100 000 [1]. Može se javiti u bilo kom životnom dobu i pojedini endokrini organi mogu biti zahvaćeni autoimunskim procesom bilo kojim redom. Bolest se najčeće javlja kod osoba od 30 do 40 godina, sa tri puta većom učestalošću kod žena [2]. Kod oko polovine slučajeva prva manifestacija sindroma je adrenalna insuficijencija. Procenjuje se da oko 50% obolelih od nadbubrežne insuficijencije tokom života razvija još neku pridruženu autoimunsku bolest. Poznata je povezanost HLA haplotipova DR3 i B8 kao važnih faktora rizika od razvoja APS 2. U ostale bolesti koje se mogu javiti udruženo sa APS 2 spadaju hipergonadotropni hipogonadizam, autoimunski hipofizitis, hronični atrofični gastritis, hronični hepatitis, reumatoidni atritis, trombocitopenijska purpura, vitiligo i neurološke bolesti poput mijastenije gravis. Mijastenija gravis se javlja kod manje od 1% obolelih od APS 2 i do sada je opisano u literaturi svega nekoliko slučajeva.

Prikaz bolesnika

Bolesnica, stara 49 godina, premeštena je iz Zdravstvenog centra (ZC) u Vrbasu, gde je lečena u junu 2006. godine sa dijagnozom akutne nadbubrežne insuficijencije postavljenom na osnovu slabosti, malaksalosti, upornog povraćanja i proliva, te prisutnog elektrolitskog disbalansa. Nivo serumskog kortizola iznosio je 36,9 nmol/L (normalno 138–690 nmol/L), vrednosti serumskog aldosterona 2 ng/dL (normalno 1–16), dok su vrednosti serumskog kalijuma (K) iznosile 6,8 mmol/L, serumskog natrijuma (Na) 133 mmol/L, uprkos prolivima i povraćanju koji su trajali dva meseca pred prijem u Zdravstveni centar u Vrbasu, pri čemu je prethodno ambulantno isključena infektivna priroda proliva negativnim nalazom koprokultura. Vrednosti uree pri prijemu iznosile su 8,9 mmol/L (normalno do 8,2 mmol/L). Tokom hospitalizacije u Vrbasu bolesnica je lečena preparatima kortikosteroida u dozi 3 × 80 mg *iv* metilprednizolona na dan, infuzionim rastvorima, blokatorima protonske pumpe i antibioticima. Bolesnica je premeštena u Kliniku za endokrinologiju Kliničkog centra (KC) Vojvodine zbog nepotpunog oporavka od primenjene terapije, kao i razvoja paranoidnih ideja i negativističkog stava prema lečenju. Dan pre premeštaja bolesnice nastupila je psihomotorna uznemirenost i odbijala je dalje lečenje zbog straha od lekara. Heteroanamnestički, saznali smo da je pre nekoliko meseci bolesnica prestala da uzima hranu.

Objektivno, na prijemu, bolesnica je bila afebrilna, eupnoična, tahikardična, u stanju stupora, otežane verbalne komunikacije, nepokretna, braneći se pri pokušaju podizanja kapaka zbog pregleda zenica. Arterijski pritisak bio je 100/60, puls oko 80 u minuti, nalaz u abdomenu uredan.

Zbog stanja bolesnice konsultovan je konzilijarni neurolog koji je indikovao komputerizovanu tomografiju (KT) endokranijuma, a na načinjenom KT snimku osim mikroishemijskih promena nisu registrovane druge patološke promene. Neurološki nalaz bio je: kranijalni nervi bez ispada, vrat bez meningealnog podražaja, motornotetivni refleksi simetrični, *knee power output* (KPO) je obostrano u plantarnoj fleksiji, znak Babinskog negativan. Cerebelarni i ekstrapiramidni znaci nisu bili ispoljeni, ali je saradnja bila insuficijentna (bolesnica tada nije kontrolisala sfinktere). Nalaz KT pregleda mozga nije odgovarao kliničkoj slici.

Neurolog je savetovao kontrolu psihijatra, a neurološku kontrolu po potrebi. Konzilijarni psihijatar konstatovao je stanje stupora zbog zaklopljenih očiju, otežane komunikativnosti (bolesnici je maternji jezik mađarski) i negativističkog stava i savetovao je dalje praćenje bolesnice bez farmakoterapije.

Laboratorijski nalazi bili su sledeći: hemoglobin 95 g/L, glukoza u krvi našte i 2 sata nakon jela 4,1 i 5,5 mmol/L; alanin aminotrasferaza (ALT) 106 J/L, K^+ 4,04 mmol/L, Na^+ 148 mmol/L, hloridi 113 mmol/L, ukupni proteini 55 g/L; ukupni holesterol 4,21 mmol/L. Ostali laboratorijski nalazi bili su uredni.

Pregled abdomena primenom KT otkrio je prisustvo umerene hipotrofije desnog bubrega. U projekciji nadbubrega nisu bile viđene ekspanzivne, niti infiltrativne lezije.

Tokom hospitalizacije kod bolesnice je dijagnostikovan i primarni hipotireoidizam na koji je posumnjano zbog opšte slabosti i psihičkog stanja bolesnice**.** Sumnja je potvrđena i laboratorijskim nalazom tireoidnih hormona u krvi: slobodni tiroksin 4 – *free thyroxine* 4 (FT4) 8,5 pmol/L (normalno 10,5–23,1 pmol/L), tirostimulišući hormon (TSH) preko 100 mIU/L, nivoi antitiroid-specifičnih peroksidnih antitela (anti TPO At) nisu rađeni iz tehničkih razloga, a načinjeni su tokom druge hospitalizacije. Bolesnica je lečena parenteralnim preparatima kortikosteroida (metilprednizolon 120 mg dnevno, uz postepeno smanjivanje doze), antibioticima uz infuzije glukoze 5% i 0,9% natrijum-hlorida. Već posle nekoliko dana terapije bolesnica se osećala znatno bolje, a započeta je i postepena supstitucija preparatima l-tiroksina, nakon adekvatne glikokortikoidne supstitucije.

Nivoi serumskog kortizola već na početku hospitalizacije bili su 1190 pmol/L (terapija kortikostreoidima u ZC Vrbas).

Nalaz testa sa vodom koji je načinjen pre završetka hospitalizacije u KC Vojvodine bio je uredan.

Otpuštena je nakon 14 dana bolničkog lečenja u poboljšanom opštem stanju, pokretna, komunikativna, normotenzivna sa predlogom za dalju peroralnu terapiju: tablete hidrokortizona od 20 mg ½ ujutro, levotiroksin natrijuma 75 μg 1 × 1 na dan, gvožđe (II) sulfata od 105 mg, 2 × 1, folne kiseline od 5 mg 2 × 1, famotidina 20 mg, 1 uveče, klonazepama 2 mg ¼ + 0 + ½ na dan.

Tokom jula 2007. godine bolesnica je imala epizodu prolazne oduzetosti ekstremiteta uz prisutan otežan govor i hod. Načinjena je magnetna rezonanca (MRI) endokranijuma ambulantno, a nalaz je bio uredan. Bolesnica je tokom 2007.

godine bila na gorenavedenoj supstituiconoj terapiji preparatima hidrokortizona i l-tiroksina.

U januaru 2008. bolesnica je rehospitalizovana u teškom opštem stanju sa znacima opšte slabosti, osećajem nemoći, ptozom kapaka, otežanim gutanjem, te nemogućnošću hoda i slabije komunikativnosti dva meseca pred prijem. Na prijemu je bila afebrilna, eupnoična, normokardična. Na plućima i srcu nalaz je bio uredan. Arterijski pritisak bio je 130/80 mmHg, puls oko 80 u minuti. Abdomen je bio urednog nalaza.

Laboratorijskim ispitivanjima načinjenim pri prijemu utvđene su normalne vrednosti serumskog kortizola u krvi – 502 noml/L, vrednosti FT4 u granici normale – 14,2 pmol/L, dok su vrednosti TSH bile povišene (22,3 mIU/L).

Težina stanja bolesnice nije se mogla objasniti prisutnim supkliničkim hipotireoidizmom, te su izvršena i dopunska opšta i imunološka ispitivanja. Konsultovan je konzilijarni neurolog koji je ustanovio da je bolesnica svesna, urednog govora, sa prisutnom ptozom kapaka obostrano (pri pregledu bulbomotorike postojala neadkvatna saradnja, te istu nije bilo moguće ispitati), zenice su bile jednake, meningealni znaci negativni, vrat slobodan, prisutni znaci flakcidne tripareze bili su izraženije u distalnim segmentima ekstremiteta. Slabost na donjim ekstremitetima bila je izraženog stepena te bolesnica nije mogla da hoda.

U laboratorijskim nalazima antitela IgM i IgG na *Borrelia burgdorferi* bila su negativna. Anticitoplazmatska antitela i ANCA – antinuklearni faktor i antinuklearna antitela, kao i antimitohondrijalna, antiparijetalna, antiglatkomišićna, antisrčana i antikardiolipinska antitela bila su negativna. Vrednosti AC – IgG 2 U/mL, AC – IgM 8 U/mL, C3 1,73 g/L, C4 0,44 g/L, CPK 24 U/L, Hgb 134 g/L, urea 5,1 mmol/L, kreatinin 112 μmol/L, *acidum uricum* 370 μmol/L, folna kiselina 38,8 nmol/L, elektroliti (mmol/L): K^+ 4,2, Na^+ 144, Cl^- 109, Ca^{++}1,18, Mg^{++} 0,88, P^{++} 1,28, Fe^{2+} 10,4 μmol/L. Elektroforeza serumskih proteina bila je uredna. Ukupni holesterol bio je 5,73 mmol/L, ukupni trigliceridi 1,46 mmol/L. glikemija našte i 2 sata nakon jela 5,4 i 4,8 mmol/L.

Rezultati vezani za endokrinološka ispitivanja iznosili su: FT4 14,2 pmol/L, TSH 22,38 mIU/L, auto antitela (At) na TSH receptore: 4,7 U/L (normalno do 2U/L), anti TPO At 48,8 IU/mL (normalno do 5,6 IU/mL), kortizol u 8 sati 502 nmol/L.

Kompjuterizovanom tomografijom endokranijuma u moždanom parenhimu nađene su promene gustine, znakova intrakranijalne hemoragije, ni tumorskih infiltracija. Na kostima lobanje nisu se videle promene. Bazalne cisterne i komorni sistem bile su u granicama normale.

Transkranijani dopler vertebralnih arterija i karotidnog sliva nije otkrio znakove manifestnog hemodinamskog poremaćaja.

Elektromiografski nalaz ukazivao je na postojanje difuzne aksonske lezije koja je bila težeg stepena distalno, bez znakova aktuelizacije. Registrovano je postojanje difuzne mijelinske lezije. U celini, nalaz je predstavljao sliku polineuropatije izraženog stepena. Na sistemima *n. ulnarisa* obostrano i *n. medianusa* desno načinjen je test neuromišićne transmisije koji je ukazao na postojanje poremećaja neuromišićne transmisije na svim ispitanim mišićima. U cilju potvrde eventualne mijastene geneze trebalo je uzeti antitela na acetilholinske (ACh) receptore. Nalaz vizuelnih evociranih potencijala (VEP) ukazivao je na postojenje smetnji provođenja obostrano prehijazmalno, izraženo pri desnostranoj stimulaciji u ispitivanim optičkim putevima. Ispitivanja evociranih potencijala moždanog stabla (BAERs) i somatosenzornih evociranih potencijala (C-SEP i L-SEP) bila su u granicama normale. Takođe, načinjen je neostigminski test na koji je bolesnica izrazito pozitivno reagovala – samostalno prohodala.

Kompjuterizovana tomografija medijastinuma (sa kontrastom) pronašla je da nema znakova aktivne infiltracije parenhima. Limfni čvorovi aksila, medijastinuma i oba hilusa nisu bili značajno uvećani. U prednjem medijastinumu nije bilo patoloških promena gustine. Srčani masiv nije bio uvećan.

U dogovoru sa konzilijarnim neurologom nakon adekvatne hormonske suptitucije bolesnica je premeštena u Kliniku za neurologiju KC Vojvodine gde je dovršena započeta neurološka dijagnostika i terapija. U objektivnom neurološkom nalazu uočeni su diskonjugovani pokreti očnih jabučica sa dvoslikama. Bila je prisutna hipomimija i slabost muskulature vrata težeg stepena, gutanje otežano, gruba motorna snaga ekstremiteta bila je snižena, bolesnica nije mogla da zadrži ekstremitete u antigravitacionom položaju, tetivni refleksi bili su difuzno sniženi, bez patoloških refleksa i ispada senzibiliteta.

Antitela na acetilholinske receptore bila su pozitivna – 9,8 nmoL.

Po uvođenju antiholinesterazne i kortikosteroidne terapije (tbl. piridostigmina od 60 mg, 3 × 2, amp. prednizona *pp*) došlo je do postepenog oporavka bulbomotora, mišića žvakača kao i snage ekstremiteta, uz manje fluktuacije motorne slabosti. Bolesnici je pri otpustu bulbomotorika bila očuvana, snaga žvakača uredna, prisutna umerena slabost fleksora vrata, uz blaži motorni deficit distalnih delova sva četiri ekstremiteta, kao i proksimalnih grupa mišića nogu. Bolesnica pri otpustu bila samostalno pokretna. Otpuštena je sa sledećom terapijom: tbl. piridostigmin 60 mg 3 × 2, tbl. prednizona od 60 mg svaki drugi dan, tbl. hidrokortizona od 20 mg ¾ + ¼ + 0, tbl. levotiroksina-Na, 75 μg × 1, tbl. ranitidina 150 mg, 2 × 1.

Na sledećim neurološkim ambulantnim kontrolama bolesnica je bila nepromenjenog neurološkog nalaza do decembra 2008. godine, kada je rehospitalizovana zbog pogoršanja obostrane ptoze kapaka, otežane fonacije, artikulacije i gutanja, mišićne slabosti fleksora vrata i mišića nogu u celini. Tretirana je preparatima kortikosteroida i ciklosporina (tbl. hidrokortizona 20 mg, 1 + ½ + ½, tbl. ciklosporina 50 mg, 3 × 1, imunoglobulini *iv* 120 g ukupno, podeljeno u četiri dnevne doze uz supstituciju preparatima levotiroksina-Na, 75 mg na dan). Na primenjenu terapiju došlo je do oporavka mišićne snage na svim grupama mišića, uz zaostajanje blaže slabosti proksimalnih mišića nogu.

Nakon hospitalizacije bolesnici je predložena operativno uklanjanje tumusa koje je i izvršeno tokom marta 2009.

godine u Institutu za grudnu hirurgiju u Sremskoj Kamenici (patohistološki nalaz: timusno tkivo u involuciji).

Kod bolesnice je na kontroli 2010. godine bila prisutna samo blaga slabost proksimalne muskulature nogu. Bolesnica je na terapiji tbl. azatioprina 50 mg, 1 × 1 uveče, tbl. piridostigmina, 2 × 60 mg, uz terapiju tbl. hidrokortizona 30 mg + 10 mg + 0, tbl. fludrokortizona 1 × 1, tbl. levotiroksin-Na 75 μg, 1 × 1.

Diskusija

Kod prikazane bolesnice početna bolest bila je insuficijencija nadbubrega, a neposredno nakon prijema u bolnicu otkriveno je i prisustvo autoimunskog hipotireoidizma (povišena anti-TSH At, povišena anti TPO At, poremećeni nivoi TSH, FT4, FT3). Iako je nadbubrežna insuficijencija verifikovana na osnovu kliničke slike, laboratorijskih vrednosti hormona (niske vrednosti kortizola, aldosterona, vrednosti elektrolita), prisustvo autoimunskog procesa nadbubrega nije verifikovano prisustvom antitela na 21-hidroksilazu nadbubrega, pošto ovaj imunoesej nije dostupan u našoj zemlji. Indirektni dokaz o autoimunskom procesu kao uzročniku nadbubrežne insuficijencije predstavljao je morfološki normalan izgled nadbubrega na snimku KT nadbubrega, uredan nalaz na plućnom parenhimu, kao i odsustvo drugih bolesti koje mogu dovesti do nadbubrežne insuficijencije. Betterle i sar. [3] našli su, takođe da je kod velikog procenta obolelih od autoimunske nadbubrežne insuficijencije nalaz KT nadbubrega uredan.

Udruženost mijastenije gravis sa autoimunskim bolestima odavno je poznata [4]. Najčešće endokrino autoimunsko oboljenje koje se javlja udruženo sa mijastenijom gravis je autoimunska bolest štitaste žlezde. Javlja se kod oko 7,3% obolelih od mijastenije gravis [5]. Nasuprot ovome, podaci o udruženoj pojavi mijastenije gravis u okviru APS 2 nedostaju čak i kod autora koji su lečili veliki broj obolelih od APS 2 [5]. U nama dostunoj literaturi opisano je svega nekoliko obolelih od mijastenije gravis i APS 2 [6]. Verovatno je da u razvoju poliglandularne autoimunosti leži dubok poremećaj u celularnom imunitetu, a za autoantigene endokrinih žlezda smatra se da imaju ključnu ulogu u pokretanju autoimunskog procesa, mada, unutarćelijska lokalizacija ovih antigena budi sumnju u isključivu odgovornost ovih antigena za pokretanje autoimunskog procesa [5]. Dokazana je nedovoljna aktivnost supresorskih T-limfocita prisutna u endokrinim autoimunskim bolestima kao i kod obolelih od mijastenije gravis. Prema novijim teorijama, kod genetski predisponiranih osoba nakon pokretanja spoljnim agensom dolazi do razvoja autoimunskog procesa zavisnog od Th-2 limfocita koji je primarno humoralnog porekla, a samo delom lokalan. Imunski proces postiže hronicitet najverovatnije zbog deficijentne aktivnosti T-supresorskih limfocita [7].

Pri drugoj hospitalizaciji težina stanja bolesnice, pogotovo kada je reč o mišićnoj slabosti, nije mogla biti objašnjena endokrinim uzrocima, samo verifikovanim supkličkim hipotireoidizmom sa normalnim vrednostima slobodnih tireoidnih hormona i kortizola. Sprovedena ispitivanja nisu potvrdila prisustvo imunološkog sistemskog oboljenja, kao ni sistemskog mišićnog oboljenja. Nivoi vitamina B12 i folne kiseline pokazivali su normalne vrednosti u serumu. Postavljena je sumnja na prisutvo klinički manifestne mijastenije gravis koja je trajala izvestan vremenski period, najverovatnije još pre prijema bolesnice prvi put na hospitalno lečenje, na šta ukazuju simtomi otežanog podizanja kapaka, kao i otežanog gutanja. Primena kortikosteoridne terapije u sklopu lečenja nadbubrežne insuficijencije maskirala je kliničku sliku mijastenije, odnosno, delovala je u izvesnoj meri terapijski u odnosu na mijasteniju gravis. Gubitak apetita u sklopu Adisonove bolesti i otežano gutanje koje se javlja u okviru mijastenije mogu uzrokovati zabunu u vezi sa gubitkom telesne mase i malaksalosti, a otežana komunikacija, zbog otežanog govora, činila je anamnezne podatke manje preciznim. Nespecifičan početak bolesti sa mogućim preklapanjem simptoma više autoimunsko uslovljenih oboljenja postavlja pitanje da li je jedna autoimunska bolest u okviru APS 2 prethodila ostalim ili su se razvijale istovremeno, što je verovatnije kod prikazane bolesnice. Mijastenija gravis dokazana je kliničkom slikom, pozitivnim piridostigminskim testom, nalazom elektromiografije mišića donjih ekstremiteta, prisustvom antitela na acetilholinske receptore, te kliničkim oporavkom bolesnice na primenjenu terapiju. Kompjuterizovana tomografija toraksa nije potvrdila prisustvo tumora u prednjem medijastinumu.

Terapija autoimunskog poliglandularnog sindroma u osnovi se ne razlikuje od supstitucione terapije za pojedine endokrine organe zahvaćene autoimunskim procesom, sa napomenom da supstituciona terapija tireoidnim hormonima mora da usledi nakon već primenjene glikokortikoidne terapije jer, u suprotnom, može provocirati pogoršanje neprepoznate adrenalne insuficijencije. U odnosu na praćenje ovih bolesnika treba imati u vidu da su osobe sa povišenim titrom autoantitela prema određenom endokrinom organu u povećanom riziku od razvoja klinički manifestne bolesti, te zahtevaju doživotno praćenje. Prikazana bolesnica podseća da se širok spektar endokrinih i neendokrinih autominih obolenja može javiti zajedno kod istog bolesnika ili nakon određenog proteklog vremenskog perioda u toku života. Ne treba zaboraviti da su supkliničke forme bolesti još učestalije od potpuno ispoljenih formi oboljenja, kao i to da često ostaju neprepoznate. Oko polovine bolesnika sa APS 2 ima rođake koji boluju od autoimunskih oboljenja [8, 9], ali se pored genetske predispozicije za HLA položaj moraju posmatrati i faktori sredine koji dovode do nastanka obolenja, kao i ne-HLA mesta koja mogu biti povezana sa razvojem ove bolesti [10]. Prisustvo autioantitela na TSH receptore, takođe, ukazuju na potrebu pažljivog praćenja nivoa TSH i slobodnih tireodnih hormona, kao i na mogućnost prelaska hipotireoidizma u hipertireodizam u okviru Gravesove bolesti kao dela APS 2.

Zaključak

Prikazana bolesnica ukazuje na neophodnost detaljnog ispitivanja bolesnika sa autoimunskim oboljenjem zbog mogućnosti udruženosti sa drugim autoimunskim oboljenjima. Kod prikazane bolesnice postojala je udruženost autoimunskog poliglondularnog sindroma tipa 2 sa mijastenijom gravis.

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CASE REPORT

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External ear canal cholesteatoma after ventilation tube insertion and mastoidectomy

Holesteatom spoljnjeg slušnog kanala posle umetanja aeracione cevčice i mastoidektomije

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Abstract

Introduction. Etiopathogenetically, there are two types of chollesteatomas: congenital, and acquired. Numerous theories in the literature try to explain the nature of the disease, however, the question about cholesteatomas remain still unanswered. The aim of the study was to present a case of external ear canal cholesteatoma (EEC) developed following microsurgery (ventilation tube insertion and mastoidectomy), as well as to point ant possible mechanisms if its development. **Case report.** A 16-year-old boy presented a 4-month sense of fullness in the ear and otalgia on the left side. A year before, mastoidectomy and posterior atticotomy were performed with ventilation tube placement due to acute purulent mastoiditis. Diagnosis was based on otoscopy examination, audiology and computed tomography (CT) findings. CT showed an obliterative soft-tissue mass completely filled the external ear canal with associated erosion of subjacent the bone. There were squamous epithelial links between the canal cholesteatoma and lateral tympanic membrane surface. They originated from the margins of tympanic membrane incision made for a ventilation tube (VT) insertion. The position of VT was good as well as the aeration of the middle ear cavity. The tympanic membrane was intact and of normal appearance without middle ear extension or mastoid involvement of cholesteatoma. Cholesteatoma and ventilation tube were both removed. The patient recovered without complications and shortly audiology revealed hearing improving. Follow-up 2 years later, however, showed no signs of the disease. **Conclusion.** There could be more than one potential delicate mechanism of developing EEC in the ear with VT insertion and mastoidectomy. It is necessary to perform routine otologic surveillance in all patients with tubes. Affected ear CT scan is very helpful in showing the extent of cholesteatoma and bony defects, which could not be assessed by otoscopic examination alone.

Key words:
cholesteatoma; tympanic membrane, perforation; ear, external; tomography, x-ray computed; reoperation.

Apstrakt

Uvod. Holesteatom spoljnjeg slušnog hodnika retko je otološko oboljenje. Etiopatogenetski, razlikuju se dve vrste holesteatoma: kongenitalni i stečeni. Mada u literaturi postoje brojne teorije koje pokušavaju da objasne prirodu nastanka oboljenja, još uvek je otvoreno pitanje zašto se holesteatom javlja. Cilj rada bio je da se prikaže slučaj pojave holesteatoma spoljnjeg slušnog hodnika posle mikrohirurške intervencije (insercija aeracione cevčice i mastoidektomija) i da ukaže na moguće mehanizme njegovog razvoja. **Prikaz bolesnika.** Bolesnik, star 16 godina, ispitivan je i lečen zbog recidivirajućeg akutnog otitisa. Godinu dana kasnije, posle umetanja aeracione cevčice i mastoidektomije, bez simptoma ponovne pojave infekcije, u levom spoljnjem slušnom hodniku nađen je holesteatom. Na presecima kompjuterizovane tomografije (KT) temporalne kosti utvrđeno je da holesteatom u celini ispunjava lumen spoljnjeg slušnog hodnika i da nema patološkog procesa u šupljinama srednjeg uva. Holesteatom je nastao zbog odlaganja predviđenih kontrolnih pregleda. Kod bolesnika je urađena reviziona operacija (mastoidektomija i uklanjanje aeracione cevčice), pri čemu je prikazani holesteatom spoljnjeg slušnog hodnika u celini odstranjen. Na

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kontrolnim pregledima lokalni nalaz bio je uredan, sluh normalan, a KT nalazi nisu ukazali na pojavu recidiva oboljenja. **Zaključak.** Pojava holesteatoma prikazanog bolesnika značajna je pri razmatranju patogeneze ovog oboljenja. Holesteatom je nastao posle umetanja aeracione cevčice, najverovatnije proliferacijom epitela sa ivica incizije bubne opne u lumen spoljnjeg slušnog hodnika. Međutim, mehanizami ovog procesa su kompleksni i multifaktorijalne prirode.

Ključne reči:
holesteatom; bubna opna, perforacija; mastoiditis; uvo, spoljašnje; tomografija, kompjuterizovana, rendgenska; reoperacija.

Introduction

First report of epithelial debris accumulation in the external ear canal was made in 1850 by Toynbee [1]. The term external ear canal cholesteatoma (EEC) was introduced in 1893 by Scholefield [2]. We presented a case of EEC after a ventilation tube (VT) insertion and mastoidectomy. It is a demonstration of a direct relationship between the use of a VT after mastoidectomy and the later development of canal cholesteatoma.

Case report

A 16-year-old boy presented with a 4-month history of a sense of fullness in the left ear and occasionaly otalgia on the same side. A year before admission the patient was submitted to surgery on the left ear due to acute purulent mastoiditis. Mastoidectomy and posterior atticotomy were performed with a ventilation tube (Tübingen-gold) placement. The patient was treated with antibiotics and his recovery was uneventful. On recall examination, microotoscopy was normal, as well as auditive and vestibular function.

On admission otoscopy examination revealed a complete obliteration of the left external ear canal (Figure 1).

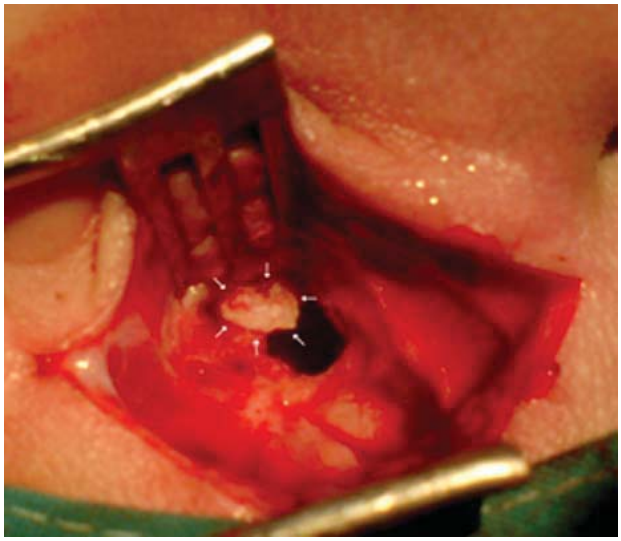

Fig. 1 – The external auditory ear canal with obliterative cholesteatoma

Pure tone audiogram showed left sided medium conductive hearing loss. There was no otorrhea or vertigo. Threedimensional multislice computed tomography (3D MSCT) showed a circumferential soft-tissue mass completely filling the external ear canal with associated erosion of the subjacent bone (Figure 2). A ventilation tube was clearly seen, with well aeration. A tympanic membrane was intact and of normal appearance without middle ear extension or mastoid involvement of cholesteatoma (Figure 3). The middle ear ossicules were also unaffected.

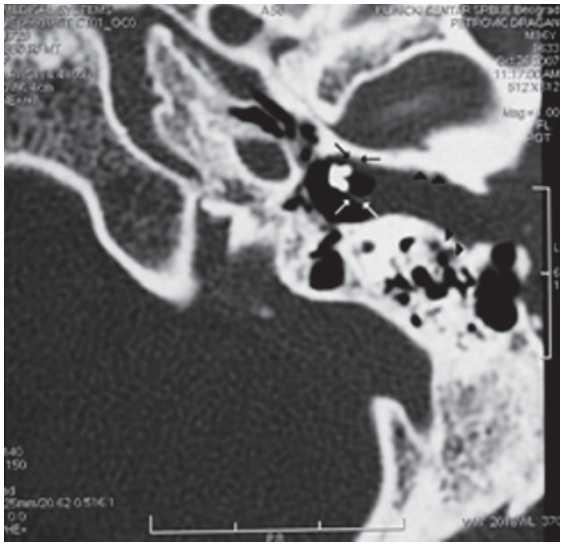

Fig. 2 – Axial temporal bone computed tomography (CT) image show the soft-tissue mass filling the external ear canal, the superior (black arrowheads) and inferior (white arrowheads) links with ventilation tube insertion; the mass has caused erosion of the canal walls (black arrows)

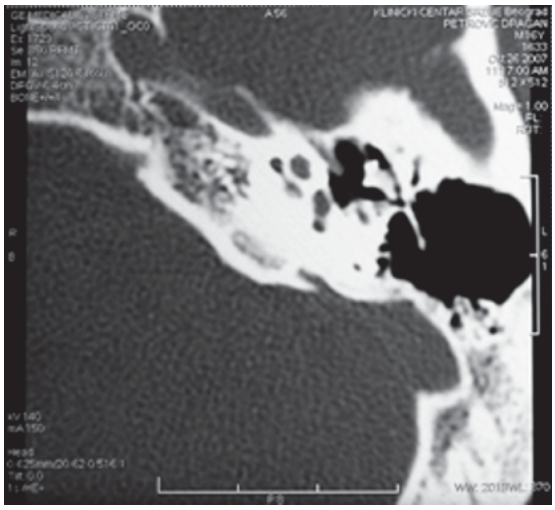

Fig. 3 – Coronal temporal bone computed tomography (CT) shows removal of mastoid cells, normal position of a ventilation tube and intact middle ear cavity

The patient underwent revision mastoidectomy surgery and extirpation of a VT. Intraoperatively, we confirmed normal findings in mastoid and middle ear cavities (Figure 4). Firstly, obliterative canal cholesteatoma was excised *en block* and then we removed a VT. From the margins of the tympanic membrane incision we noted a proliferation squamous epithelium remnants skipted laterally forming a connection with cholesteatoma. In the ear canal we also found the signs of bony wall erosion, partcularly of the anterior wall. A defect of meatal skin was excised and the irregular eroded area was drilled. The bone turned to normal and healthy canaloplasty. Gross examination revealed an ovoid white shaped mass. Histopathology revealed stratified squamous keratinizing epithelial sac (matriks) with poor by developed perimatrix. Postoperatively, the patient was well. The place of tympanic membrane incision and meatal skin were spontaneosly healed within one week. Audiology showed improving in hearing with minimal loss around 20 dB. The patient was reviewed regularly and 2 years later showed no signs of the disease.

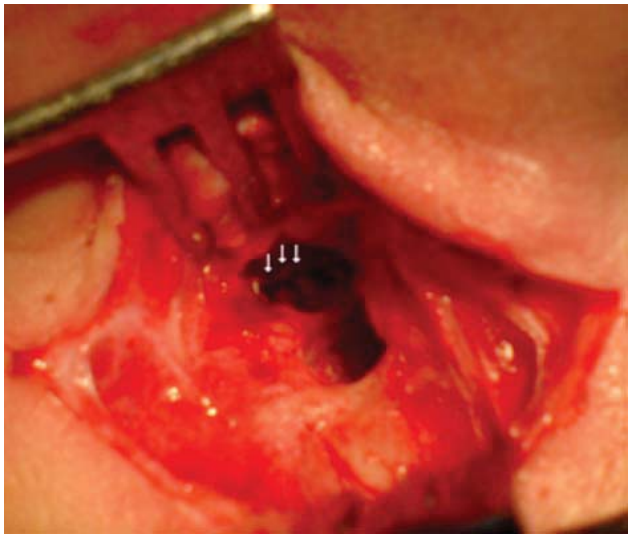

Fig. 4 – The tympanic membrane is normal and anterior bony wall erosion (arrowheads) of the external ear canal is present after removing cholesteatoma (note the intact canal wall after mastoidectomy)

Discussion

There are several well-known mechanisms of developing of EEC following different otologic surgeries. Cholesteatomas can occur in the anterior sulcus as a complication of lateral graft tympanoplasty [3]. If the epithelium is trapped under the vascular strip, the cholesteatoma will form more laterally in the canal, along the posterior wall [4]. Secondary EEC has been seen as a postoperative complication after mastoid surgery and may result from entrapment of squamous epithelial debris during the healing process [5].

Cholesteatoma is considered a complication of a VT placement when developes behind an intact drum or next to a perforation at or near the site of the tube insertion, in the mesotympanum or hypotimpanum [6]. There is some controversy regarding the development of cholesteatoma following middle ear VT placement. Cholesteatoma may arise as a direct complication of a VT placement in a retraction pocket in an atrophic and flaccid area in the drum that progresses to the point of debris accumulation, as a result of shedding and implantation of epithelial cells into the middle ear or due to ingrowth of squamous epithelium from the perforation margin to undersurface of the drum [7, 8].

Theoretically, conditions for the development of secondary cholesteatoma are perfect both when a VT is in place and when a perforation or atrophic scar remains at the implantation site. Because such cholesteatomas have been not encountered following myringotomy alone or mastoidectomy procedure we cannot accept seeding or implantation theories. Causative factor in our case could be reverse epithelial ingrowth from the incision margins to the undersurface of the drum, directed and enhanced by the tube's flanges. Another possibility was mastoid infection stimulating changes in ear drum. Indication of mastoidectomy and of tube placement was usually chronic persistent middle ear effusion unresponsive to medical therapy. But in our patient a VT insertion was performed due to acute supurative mastoiditis. A bacterial infection initially could cause erosion of the epithelial layer and granulation tissue at the place of a VT and be partly related to later canal accumulation of keratin debris. But it is contradictory to EEC developed in an ear without recurrent episodes of otorrhea in a so-called "dry" ear without infection, as in case we presented. Most areas of focal atrophy or retraction are cosmetic and nonprogressive, related to the absence of a fibrous middle layer of the tympanic membrane at intubation site [9]. This becomes problematic only rarely if progresses to a retraction pocket and onward to cholesteatoma (more common with longterm tubes) [10]. As spontaneous extrusion did not occurr, and because ventilation was sufficient, intention to remove the tube was not realised. We assumed that cholesteatoma may not develop either in a retraction pocket, in an atrophic scar as the tympanic membrane was rather normal. We also hypothesed that as mastoidectomy have performed, the pattern of epithelial migration of external ear canal to outside could be influenced by restoring the normal ventilation of the middle ear. Slower migration rates have already been demonstrated in the inferior wall in patients with ECC and cholesteatoma we presented was mostly at that site. It was similarly suggested that it could be explained by hypoxic conditions due to poor blood supply [11].

The main symptom was progressive conductive hypoacusis and it may be related to occlusion of the external canal by cholesteatoma plug in obliterative cholesteatoma of ECC [12], but many cases can be remarkably silent or even asymptomatic. The invasion of squamous tissue with periostitis may explain chronic dull pain experienced by our patient. However, acute severe pain found more frequent in keratosis obturans [13]. A preoperative high-resolution temporal bone CT is helpful whenever the surgeon suspects EACC eroding into adjacent anatomic structures. Erosion involving more than one EEC wall is typical [14] but EEC could be more extensive than that suggested by clinical findings [15]. For localized small

lesions, treatment consists of frequent cleaning with debridement of necrotic tissue. Deeper pockets can be managed with canaloplasty by removing diseased skin and bone and exteriorizing the recess.

Conclusion

Although tympanostomy tubes are safe and efficacious for most patients with refractory otitis media or mastoiditis, they are associated with significant sequelae like cholesteatoma development. EEC is a rare entity after VT insertion with characteristic imaging and clinical features but different variables may influence its development. Exact fine mechanisms of cholesteatoma forming in the external ear canal near a tube placement site are still unknown. It is necessary to perform routine otologic surveillance in all patients with tubes. Affected ear CT scan is very helpful in showing the extent of cholesteatoma and bony defects, which could not be assessed by otoscopic examination alone.

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CASE REPORT

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Atypical presentation of cystic fibrosis – obese adolescent with hypertension and pseudo-Bartter's syndrome

Atipična prezentacija cistične fibroze – gojazni adolescent sa hipertenzijom i pseudo-Barterovim sindromom

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Abstract

Introduction. Infants with cystic fibrosis may fail to thrive despite recommended caloric intake because of electrolyte disurbances caused by salt depletion resulting in hypochloremic metabolic alkalosis or pseudo-Bartter's syndrome. In most patients reported symptoms began in infancy, but it may be an initial presentation of disease in a previously healthy adolescent. **Case report.** A 15-year-old boy was admitted for evaluation of recurrent episodes of malaise associated with dehydration and acute renal insufficiency. Laboratory analysis showed hypochloremic metabolic alkalosis with hyponatremia and hypokalemia. On admission the boy was obese, with body weight of 95.5 kg (> P_{97}), height 174 cm (> P_{75}), and body mass index of 31.2 kg/m^2 (> P_{95}). Physical examination was inconclusive. Blood pressure holter monitoring proved significant systolic hypertension. Routine urinalysis, protein and electrolyte levels in urine were normal. Plasma renin and aldosteron were normal. Sweat chloride concentration was 63 mmol/L. Genetic testing confirmed the diagnosis of cystic fibrosis. **Conclusion.** To our knowledge, this is the first reported case of atypical presentation of cystic fibrosis in an adolescent presented with pseudo-Bartter's syndrome and signs of obesity and hypertension. We suggest that every patient with hypochloremic metabolic alkalosis should be evaluated for cystic fibrosis.

Key words:
cystic fibrosis; diagnosis; hypertension; obesity; adolescent.

Apstrakt

Uvod. Odojčad obolela od cistične fibroze mogu zaostajati u težini uprkos dobrom kalorijskom unosu, zbog elektrolitnih poremećaja prouzrokovanih gubitkom soli, što dovodi do hipohloremijske metaboličke alkaloze ili pseudo-Barterovog sindroma. Simptomi najčešće počinju u uzrastu odojčeta, ali mogu biti inicijalna manifestacija oboljenja kod predhodno zdravih adolescenata. **Prikaz bolesnika.** Dečak uzrasta 15 godina primljen je zbog ispitivanja ponavljanih epizoda malaksalosti, udruženih sa dehidracijom i akutnom bubrežnom insuficijencijom. Laboratorijskim analizama utvrđeno je postojanje hipohloremijske metaboličke alkaloze sa hiponatrijemijom i hipokalijemijom. Dečak je bio gojazan sa telesnom masom 95,9 kg (> P_{97}), telesnom visinom 174 cm (> P_{75}) i indeksom telesne mase 31,2 kg/m^2 (> P_{95}). Fizikalni nalaz bio je uredan. Holter monitoringom arterijskog pritiska utvrđena je značajna sistolna hipertenzija. Rutinski pregled urina, nivo proteina i elektrolita u urinu, bili su normalni, kao i koncentracija renina i aldosterona u serumu. Vrednost hlorida u znoju iznosila je 63 mmol/L. Genetskim probama potvrđena je dijagnoza cistične fibroze. **Zaključak.** Prema saznanjima autora, ovo je prvi prikaz adolescenta sa atipičnom prezetacijom citične fibroze, kod koga se bolest ispoljila kao pseudo-Barterov sindrom, gojaznost i hipertenzija. Kod svakog bolesnika kome se utvrdi hipohloremijska metabolička alkaloza treba razmotriti postojanje cistične fibroze.

Ključne reči:
cistična fibroza; dijagnoza; hipertenzija; gojaznost; adolescenti.

Introduction

Cystic fibrosis (CF) is the most common autosomal recessive inherited disease in Caucasian's. From their early age patients mostly develop symptoms such as malnutrition (due to pancreatic insufficiency) and chronic suppurative lung disease. Very few patients may have mild and atypical CF phenotype. It is also well known that infants with cystic fibrosis may fail to thrive despite recommended caloric intake because of electrolyte disturbances caused by salt depletion

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resulting in hypochloremic metabolic alkalosis or pseudo-Bartter's syndrome [1]. In most patients reported symptoms began in infancy and mostly resolved with appropriate salt intake at the age of four [1, 2]. Nevertheless, it may be an initial presentation of disease in a previously healthy adolescent [3].

Case report

A 15-year-old boy was admitted for further evaluation of recurrent episodes of malaise associated with dehydration and acute renal insufficiency, requiring correction by intravenous infusion of fluids. These were first observed at the age of 10, usually occurred during summer and were provoked by physical efforts (sports training) which led to excessive sweating and malaise that sometimes resolved spontaneously. The boy was hospitalized twice in local hospital, for more severe symptoms when laboratory analysis showed hypochloremic metabolic alkalosis with hyponatremia and hypokalemia.

He was born on term and was thriving normally. From the age of 4 months, he was periodically treated with bronchodilators for acute respiratory infections associated with wheezing. Recently he was sometimes complaining on dyspnea during exercise, but he had neither chronic sputum production nor recurrent pneumonias. He had atopic dermatitis, and positive family history of atopy. Skin prick tests for inhalation allergens were negative. He did not have prolonged neonatal jaundice, meconial ileus, greasy stools or rectal prolapses. Histories of cyclic vomiting, dyspepsia, abdominal pain or dysuria were all negative.

On admission the boy was obese, with body weight of 95.5 kg (> P_{97}), height 174 cm (> P_{75}), body mass index of 31.2 kg/m^2 (> P_{95}). Auscultatory findings over the chest were normal. The rest physical examination was inconclusive.

Chest radiography showed no abnormalities. Spirometry, whole body plethysmography and impulse oscilometry showed normal values. Ergospirometry results were above normal values for age.

Pharyngeal aspirate was negative for bacterial pathogens. Blood gas analysis and oxygen saturation were within normal range. Routine urinalysis, protein and electrolyte levels in urine were also normal. Plasma renin and aldosteron were normal. Fecal elastase level was normal. Blood pressure (BP) was elevated and subsequent holter monitoring proved significant systolic hypertension. Sweat chloride concentration was elevated - 63 mmol/L.

The patient was then referred for genetic testing, which confirmed deltaF508 in one allele, but none of 29 most common mutations in our population was found on another. Therefore, a systematic scan of the whole coding regions of the CF transmembrane conductance regulator (CFTR) gene was performed, which showed that he was compound heterozygote for deltaF508 and G126D mutations. This result confirmed diagnosis of CF.

Further analyses were done. High resolution computed tomography of the chest showed very mild cylindric bronchiectasis in middle and lingular lobes. Abdominal and heart ultrasound were normal, without portal or pulmonary hypertension. Ultrasound examination of testicles showed several small cysts in the head of the both epididymis which was not uncommon finding in male CF patients.

Discussion

In classical form of Bartter's syndrome renal tubules are unable to reabsorb electrolytes, which lead to its high urine concentrations. In its several subtypes, plasma renin and aldosteron levels are elevated and blood pressure is usually within normal range, with normal sweat chloride level. Classical form of Bartter's syndrome is also characterized by the onset in early childhood [4–6]. The findings in our patient, such as late onset of symptoms, hypertension, normal renin and aldosteron levels are not indicative for classical form of Bartter's syndrome. Hypertension found in our patient is probably caused by obesity.

Pseudo-Bartter's syndrome is a metabolic disorder that can be caused by CF, but also by uncontrolled diuretic and laxative use, rigorous chloride-deficient diet, cyclic vomiting and bulimia. Symptoms may include polyuria, polydipsia, vomiting, frequent dehydration and salt craving [1–3]. In one of the largest cohort presented so far, median age at the presentation was 4 months [2].

First reported by Wagner et al. [7], G126D is a non-common missense mutation located in exon 4 of the CFTR gene. It was reported in an infant who was compound heterozygote for deltaF508 and G126D mutations, and had pancreatic insufficiency. We report an adolescent patient with the same genotype, but with mild phenotype and pancreatic sufficiency, who presented an unusual association of obesity and hypertension with pseudo-Bartter's syndrome.

It is shown that a long-term survival in CF is probably not caused by residual CFTR function, and that it is possible even with "severe" mutations, like the one found in our patient. It is proposed that mechanisms of a long-term survival include genetic modifiers and environmental factors [8, 9].

Conclusion

As to authors' knowledge, this is the first reported case of atypical presentation of CF in an adolescent presented with pseudo-Bartter's syndrome and clinical signs of obesity and hypertension. We suggest that every patient with hypochloremic metabolic alkalosis should be evaluated for CF.

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CASE REPORT

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Unilateral presentation of pseudo-Kaposi's acroangiodermatitis – a diagnostic and therapeutic challenge

Jednostrana prezentacija pseudo-Kaposijevog akroangiodermatitisa – dijagnostički i terapijski izazov

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Abstract

Introduction. Acroangiodermatitis is a rare skin disease characterised by hyperplasia of pre-existing vasculature due to venous hypertension from severe chronic venous stasis. Clinical appearance of this condition is often similar to Kaposi sarcoma and is creating serious differential diagnostic difficulties. **Case report.** A patient with acroangiodermatitis was presented and the differential diagnosis discussed. Examination of the patella of the affected area showed grayish-blue to brown infiltrates and reduced elasticity, located in the supra- and infrapatellar regions. Clinically, Kaposi's sarcoma was suspected. Histopathologically there were acanthosis and compact hyperkeratosis. The underlying papillary dermis showed fibrosis and edema. A subepidermal lobular vascular proliferation with hemosiderin deposition was also noted. This consisted of multiple newly formed capillaries, featuring small blood vessels with dilated, rounded lumina. Serologies for HIV and *Borrelia burgdorferi* were negative, as was a HHV-8 PCR in lesional tissue. Doppler analysis of the vessels of the extremities showed chronic venous insufficiency, insufficiency of *v. perforantes*, insufficiency of the Cockett II-III. No deep thromboses in the area of the shank and thigh were found. Initially, treatment consisted of clindamycin 600 mg 3 times per day, intravenously, during a 2-week period. After that the treatment was continued with prednisolone, 30 mg daily in combination with furosemide 40 mg/day, as well as lymph drainage and adequate compression therapy. The consequent clinical improvement allowed the patient to be discharged from the clinic. **Conclusion.** The most important differential diagnostic marker in distinguishing between acroangiodermatitis and Kaposi sarcoma seems to be the confirmation of the presence of genetic material of HHV-8 in the affected skin areas in patients with Kaposi sarcoma.

Key words:
acrodermatitis; skin diseases, vascular; venous insufficiency; diagnosis; diagnosis differential; drug therapy; treatment outcome.

Apstrakt

Uvod. Akroangiodermatitis spada u retko oboljenje kože koje karakteriše hiperplazija već postojeće vaskulature zbog venske hipertenzije usled ozbiljne venske staze. Klinička slika ove bolesti često ima sličnosti sa Kaposijevim sarkomom čime ozbiljno otežava diferencijalno dijagnostikovanje. **Prikaz bolesnika.** Prikazan je bolesnik sa akroangiodermatitisom uz komentar diferencijalne dijagnoze. Pregled kolena u zahvaćenom delu pokazao je sivoplave do braon infiltrate i smanjenu elastičnost u supra- i infrapatelarnim delovima. Klinički, sumnjalo se u Kaposijev sarkom. Patohistološki, nađena je akantoza i kompaktna hiperkeratoza. Papilarni dermis imao je znake fibroze i edema. Takođe, primećena je potkožna lobularna vaskularna proliferacija sa depozitom hemosiderina. Ona se sastojala iz brojnih novoformiranih kapilara, sitnih krvnih sudova proširenog, kružnog lumena. Serologija na HIV i *Borrelia burgdorferi* bila je negativna, kao i HHV-8 PCR za obolelo tkivo. Dopler krvnih sudova ekstremiteta pokazao je hroničnu insuficijenciju vena, insuficijenciju perforantnih vena i insufijenciju Cocket II-III. Nije nađena duboka tromboza u zoni potkolenice i butine. Početno lečenje baziralo se na primeni klindamicina 600 mg, tri puta dnevno, intravenozno, tokom perioda od dve nedelje. Lečenje je, zatim, nastavljeno prednisolonom 30 mg dnevno, u kombinaciji sa furosemidom 40 mg dnevno, kao i drenažom limfe i odgovarajućom kompresijom. Nastalo kliničko poboljšanje omogućilo je otpuštanje bolesnika iz bolnice. **Zaključak.** Najvažniji marker diferencijalne dijagnoze za razlikovanje akroangiodermatitisa od Kaposijevog sarkoma jeste potvrda postojanja genetskog materijala HHV-8 u zahvaćenim zonama sa Kaposijevim sarkomom.

Ključne reči:
akrodermatitis; koža, vaskularne bolesti; venska insuficijencija; dijagnoza, dijanoza diferencijalna; lečenje lekovima; lečenje, ishod.

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Introduction

Acroangiodermatitis, also called pseudo-Kaposi's sarcoma or Morbus Mali (M. Mali), is a disease that can simulate true Kaposi's sarcoma, both clinico-morphologically and histologically. Histological findings include vascular proliferations that are most frequently localized in the upper dermis with no infiltrative features [1]. Bilateral lesions are usually associated with chronic venous insufficiency, whereas unilateral lesions suggest an underlying vascular malformation. Acroangiodermatitis is a hyperplasia of pre-existing vasculature, as opposed to Kaposi's sarcoma, in which vascular proliferation is independent of the existing vessels [2] .

The concept of "acroangiodermatitis" is described in the literature in two forms: M. Mali, clinically manifested by skin alterations on the lower extremities in middle-aged to elderly patients with chronic venous insufficiency [3], and unilaterally manifested pseudo-Kaposi's sarcoma in young patients with congenital arteriovenous anastomoses of the inferior extremities, such as occurs in the Klippel-Trenaunay syndrome [4].

In addition, single reports in the literature describe the appearance of M. Mali in patients with amputated or paralyzed extremities, as well as in iatrogenically caused arteriovenous shunts (for example, in patients on hemodialysis) [5–7]. It was also thought to be secondary to a chronic trauma to some areas due to a prolonged stance in bed in the emergency room, for example [8].

Case report

A 56-year-old patient, German sailor, complained of recurring erysipelas and phlegmon in the area of the knee joint of the left leg since childhood. After adequate antibiotic therapy, the skin alterations always used to heal without any complications. Before coming to the hospital he ran a high temperature and felt heat and pain in the area of the knee joint. His general condition grew worse, so he was hospitalized.

Examination of the patella showed grayish-blue to brown infiltrates and reduced elasticity, located in the supra- and infrapatellar regions (Fig. 1a). Clinically, Kaposi's sarcoma was suspected.

Histopathologically, there were acanthosis and compact hyperkeratosis. The underlying papillary dermis showed fibrosis and edema. A subepidermal lobular vascular proliferation with hemosiderin deposition was noted. This consisted of multiple newly formed capillaries, featuring small blood vessels with dilated, rounded lumina (Fig 1b). The surrounding connective tissue featured erythrocyte extravasation and a perivascular lymphocytic infiltrate. Abnormal laboratory values included: ESR 10/16 mm, haemoglobin 13.8 g/L, antistreptolysin titre 706 E, blood sugar 6.4 mmol/L, CRP 7.8. Serologies for HIV and *Borrelia burgdorferi* were negative, as was herpes virus 8 (HHV-8) PCR in lesional tissue.

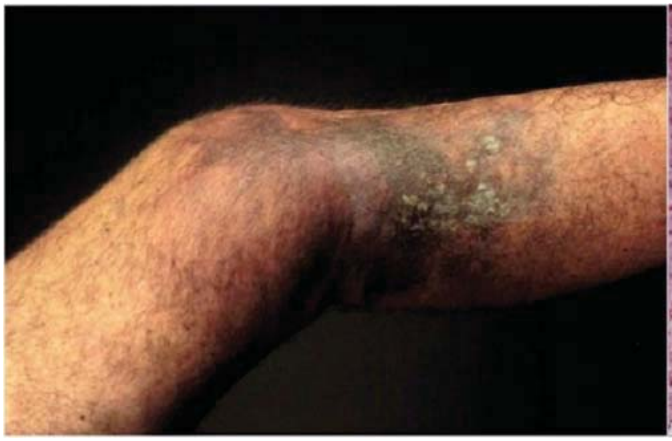

Fig. 1a – Clinical presentation of a patient with pseudo-Kaposi's acroangidermatitis

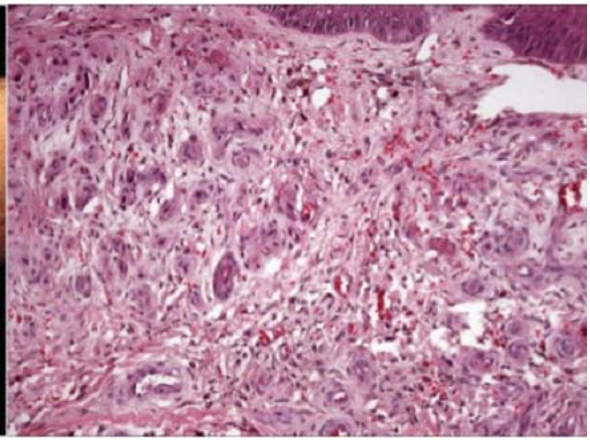

Fig. 1b – Histopathology of pseudo-Kaposi form of acroangiodermatitis with proliferative small vessels, fibrosis with some spindled cells, and hemosiderin deposition

Doppler analysis of the vessels of the extremities showed chronic venous insufficiency, insufficiency of v. perforantes, insufficiency of the Cockett II-III. No deep thromboses in the area of the shank and thigh.

Initially, the treatment consisted of clindamycin 600 mg, 3 times per day, intravenously, during a 2-week period. Due to the consequent slight improvement and to the suspicion of an inflammatory process, Kaposi's sarcoma was excluded and the diagnosis of pseudo Kaposi's acroangiodermatitis or M. Mali, complicated by erysipelas, was made. Due to severe aching and a sense of stress in the knee joint area, the treatment was initiated with prednisolone, 30 mg daily in combination with furosemide 40 mg/day, as well as lymph drainage. The consequent clinical improvement allowed the patient to be discharged from the clinic. Adequate compressions therapy was performed several weeks after the hospitalization.

Discussion

The differential diagnosis between Kaposi's sarcoma and the so-called pseudo-Kaposi's sarcoma or acroangiodermatitis of the feet is often fraught with difficulty, not only

on clinical but also on histological grounds [1]. However, considering clinical picture, histological and immunohistological findings, and also data from biomolecular analyses and electron microscopy, a clear and certain differentiation between these two diseases can be made [1, 3, 4].

In the early stages of Kaposi's sarcoma, the histological differential diagnosis may be difficult, if not impossible, since cellular atypism is not observed [1]. The histological picture includes proliferation of the vessels in the superior part of the corium and a nonspecific lymphomononuclear perivascular infiltrate. Histological picture in acroangiodermatitis may be similar. In more advanced, tumorous stages of Kaposi's sarcoma, cellular atypia becomes apparent and allows differentiation between Mali's acroangiodermatitis and true Kaposi's sarcoma. At this stage, main alterations affect the structures of the vascular wall and the endothelium [1].

In the case of acroangiodermatitis, a new formation of small, oval vessels arises and the vascular wall is thickened. In the case of Kaposi's sarcoma, lumina of the vessels are irregularly configured, and their walls are reinforced by thin endothelial cells with oval nuclei [1, 3]. The vascular formations in M. Mali are located, as a rule, at the superior part of the dermis. The Stewart-Bluefarb syndrome is an exception, because then the proliferating vessels cover the whole dermis. Kaposi's sarcoma is characterized by the generation of new vascular formations around the normal vessels. This provokes a wrong impression of newly formed or secondarily inserted normal structures in the area of the pathologic vessels [1, 3].

In both Kaposis sarcoma and M. Mali there are spindle-shaped cells, histogenetically related to fibroblasts. They predominate in the advanced stages of Kaposi's sarcoma. Augmentation of the aforesaid cells in M. Mali arises in connection with the fibrosis induced by stasis, and quantitatively they are much fewer in the number than the spindle-shaped cells in Kaposi's sarcoma. In Kaposi's sarcoma, they tend to form oblong cavernous formations, partially filled by erythrocytes. The phagocytosis of erythrocytes by the spindle-shaped cells is not characteristic for M. Mali, in contrast to Kaposi's sarcoma. Cellular atypism is observed at the late Kaposi's sarcoma stages. In spite of these morphological differences, it can be difficult to differentiate these diseases on histologic grounds alone, especially when Kaposi's sarcoma is in its early stages [9].

Immunohistological and biomolecular analyses are extraordinarily important in making the correct diagnosis. In the classic HIV-associated Kaposi's sarcoma, an expression of CD-34 antigen is observed, not only in the endothelial cells, but also in the surrounding spindle-shaped cells. The spindle-shaped cells in acroangiodermatitis are negative with respect to the CD-34 antigen [1, 10].

MS-1 high molecular weight protein (MS1-HMWP) and RM 3/1 are superficial antigens of activated macrophages. They are found not only in cases of acroangiodermatitis, but also in classic and HIV-induced Kaposi's sarcoma. It has been proved that these macrophages possess a strongly expressed angiogenic activity. But as they are observed in both diseases, they cannot be used as a differential diagnostic marker [1].

Confirmation of the presence of genetic material of HHV-8 in skin lesions of patients with Kaposi sarcoma provides the most definitive confirmation of the diagnosis [1]. HHV-8 has been found in all the forms of Kaposi's sarcoma. The lack of HHV-8 in pseudo-Kaposi's acroangiodermatitis, as was the case in our patient, demonstrates that the determination of HHV-8 by PCR is the most important and certain marker in elucidating the etiology of skin lesions when the differential diagnosis is focused on these two entities. Ideally, however, the diagnosis should be supported by histological and immunohistological analyses, if possible at all [1, 10].

Conclusion

The pathogenesis of Mali's acroangiodermatitis is not yet clear, nor are the roles of the activated macrophages in M. Mali and in Kaposi's sarcoma or the reasons for their activation. It is supposed that the increased vascular pressure in chronic venous insufficiency is capable of inducing vascular proliferation.

Important differential diagnostic markers in distinguishing between acroangiodermatitis and Kaposi's sarcoma are:

1. Confirmation of the presence of genetic material of HHV-8 (PCR – HHV or Immunhistological methods) in the affected skin areas in patients with Kaposi's sarcoma.

2. Confirmation of the presence of CD-34 antigen in the interstitial spindle-shaped cells in any form of Kaposi's sarcoma and its absence in patients with M. Mali. Immunolabelling for the CD34 antigen appears to be a valuable tool in the differential diagnosis between Kaposi's sarcoma and pseudo-Kaposi's sarcoma (interstitial cells).

3. In differentiating the two diseases, classic histological analysis and the clinic image also play some role, but are less definitive than the aforementioned methods.

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Poziv za reklamiranje u 2012. godini

U prilici smo da vam ponudimo mogućnost oglašavanja i reklamiranja proizvoda i usluga u časopisu „**Vojnosanitetski pregled**“ (VSP). To je sigurno najbolji vid i najzastupljeniji način upoznavanja eventualnih korisnika sa vašim uslugama i proizvodima.

Časopis „**Vojnosanitetski pregled**“, zvanični organ lekara i farmaceuta Vojske Srbije, naučno-stručnog je karaktera i objavljuje radove iz svih oblasti medicine, stomatologije i farmacije. Radove ravnopravno objavljuju stručnjaci iz vojnih i civilnih ustanova i iz inostranstva. Štampa se na srpskom i engleskom jeziku. Časopis izlazi neprekidno od 1944. godine do sada. Jedini je časopis u zemlji koji izlazi mesečno (12 brojeva), na oko 100 strana A4 formata, a povremeno se objavljuju i tematski dodaci (suplementi). Putem razmene ili pretplate VSP se šalje u 23 zemlje sveta. Radove objavljene u VSP-u indeksiraju: *Science Citation Index Expanded (SCIE)*, *Journal Citation Reports/Science Edition*, *Index Medicus (Medline)*, *Excerpta Medica (EMBASE), EBSCO* (preko ove baze VSP je *on line* dostupan od 2002. godine u *pdf* formatu) i *Biomedicina Serbica*.

Cene reklama i oglasa u časopisu „Vojnosanitetski pregled“ u 2012. godini su:

1.	Oglas u crno-beloj tehnici A4 formata za jedan broj	20 000,00 dinara
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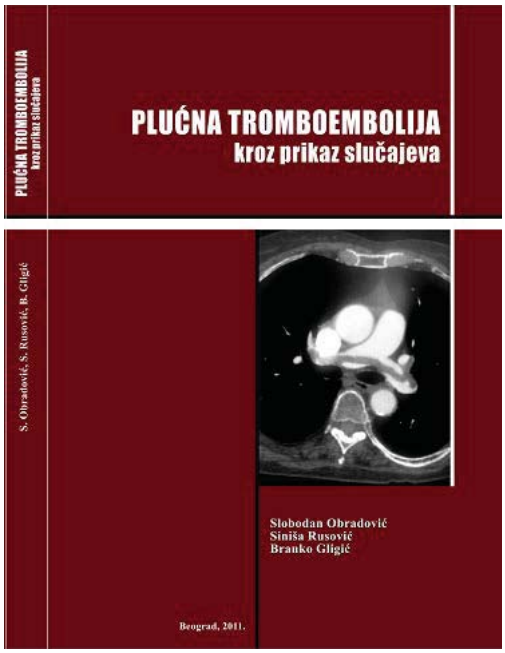
5.	Oglas u boji na koricama K3 za jedan broj	50 000,00 dinara
6.	Oglas u boji na koricama K3 za celu godinu (11-12 brojeva)	455 000,00 dinara
7.	Oglas u boji na koricama K2 i K4 za jedan broj	55 000,00 dinara
8.	Oglas u boji na koricama K2 i K4 za celu godinu (11-12 brojeva)	530 000,00 dinara

Za sva obaveštenja, uputstva i ponude obratiti se redakciji časopisa „Vojnosanitetski pregled“. Sredstva se uplaćuju na žiro račun kod Uprave javnih plaćanja u Beogradu broj: 840-941621-02 **VMA (za Vojnosanitetski pregled ili za VSP)**, **PIB 102116082**. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om) na adresu: Vojnosanitetski pregled, Crnotravska 17, 11000 Beograd; tel/faks: 011 2669 689, e-mail: vsp@vma.mod.gov.rs ili vmavsp@hotmail.com

BOOK REVIEW/PRIKAZ KNJIGE

Pulmonary thromboembolism through case reports

Original Title in Serbian: *Plućna tromboembolija kroz prikaze slučajeva*



***Authors*: Slobodan Obradović, Siniša Rusović, Branko Gligić**

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Printing: MST-Gajic Belgrade

Circulation: 500

All unsuccessful books look alike but each valuable one is innovative. Books are generally not expected to give us experience, but rather a theoretical overview. Experience, important source from which doctors learn, helps in creating ideas about differential diagnosis, improves evaluation of possible reactions to given drugs (in both typical and specific situations), assists in adjusting findings and treatment, new symptoms and signs with therapeutic answer, etc. Experience comes from practice and usually not from books. Thus, books as a source of experience are rare and valuable. Even more so, if a book focuses on a frequent, life-threatening disease, with variety of ethiopathogenic factors and plethora of clinical manifestations, such as pulmonary thromboembolism (PTE).

Such is the first book about PTE in Serbian language, which solves previous apparent discrepancy between the great importance of PTE and the lack of textbooks on it (in Serbian). The book consists of 32 case reports, presented in a pedagogic, "scholarly" manner. The stories are "real life", with a clear message (usually more than one to each case, such as the first one). The book offers 156 pages of both typical and atypical presentations of the disease (cases 1 and 2), as well as rarities, e.g. "Acute respiratory distress and PTE, in patients with ectopic adrenocorticotropic hormone secretion".

The book covers many of endless risk factors combinations, clinical characteristics, diagnostic and therapeutic dilemmas, etc, in "patients with PTE who differ so much in many aspects: they may be aged from babies to the oldest, from Paget-Schroetter syndrome in otherwise healthy sportsmen to dying patients with sepsis, respiratory failure, and heart failure."* An undeniable quality of this book is a thorough medical evaluation of each patient, as evidenced by plenty of illustrations – 251 – the result of properly applied contemporary methods, which extend far

**Hurst J. Willis.* Teaching medicine. Process, Habits and Actions. Atlanta, Georgia: Scholars Press; 1999.

beyond the cardiac frame. Authors are experts in the field, with characteristics of the "true teachers" (J. Willis Hurst). A subtle and important way of teaching the PTE is the implementation of many clinical risk scores (carefully collected and woven into the appropriate description of clinical treatment) as a routine. These scores should be percepted as necessary from doctors who take care of PTE patients.

Often diagnostic and therapeutic procedures are enriched with personal innovations. There was a danger that the book might poorly communicate with a reader, because the topic is complex. In similar situations other authors often do not clearly express what they understand perfectly. Fortunately, it did not happen and the way of storytelling is concise and fluent. Indeed, the topic is very important, both because of the high incidence of PTE, and because of the great potential to reduce mortality (and this potential is probably several times larger in comparison with acute myocardial infarction). For whom is this book written? In the first place – for curious colleagues. Because "Curiosity, or a sense of wonder, is the most important for the learning process"*.

If we bear in mind that PTE patients occur in every branch of medicine, the answer is clear – this book is for anyone who provides care for patients. There are chances for some lives to be saved by knowledge arising from the publication in front of us, and there is no greater reward for decades of careful and thoughtful work, which is built into this valuable book. To conclude, the book is an extraordinary example for how doctors can earn not only theoretical achievements, but also gain experience of inspired colleagues.

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* *Koracevic GP*. Current protocol for initial anticoagulants in pulmonary embolism: one size fits all? Am J Emerg Med 2011; 29(4): 460–2

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

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

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2. ***Apstrakt i ključne reči***

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

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

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

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Primeri oblika referenci:

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

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

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

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

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

Tabele

Sve tabele štampaju se sa proredom 1,5 na posebnom listu hartije. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Za fus-notu koristiti sledeće simbole ovim redosledom: ***, †, ‡, §, ||, ¶, **, ††, ...** Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

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Papers should be written on IBM-compatible PC, using 12 pt font, and double spacing, with at least 4 cm left margin. **Bold** and *italic* letters should be avoided. Observational and experimental articles, reviews and meta-analyses, should not exceed 16 pages (including tables and illustrations); case reports – 6; short communications – 5; letters to the Editor, reports on scientific meetings and book reviews – 2.

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Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the first author for corrections that should be returned within 3 days. Manuscripts accepted for publication are not being returned.

Preparation of manuscript

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

1. ***Title page***

a) The title should be concise but informative. Subheadings should be avoided;

b) full name of each author;

c) name and place of department(s) and institution(s) of affiliation, clearly marked by standard footnote signs.

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

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

In the **Introduction** repeat the title of the article, excluding the names of authors. State the purpose of the article and summarize the rationale for the study or observation. Give only strictly pertinent references and do not include data or conclusions from the work being reported.

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

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Examples of references:

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

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

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

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

Tables

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

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Use only standard abbreviations. Avoid abbreviations in the title and abstracts. The full term for which an abbreviation stands should precede its first use in the text.

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