YU ISSN 0042-8450

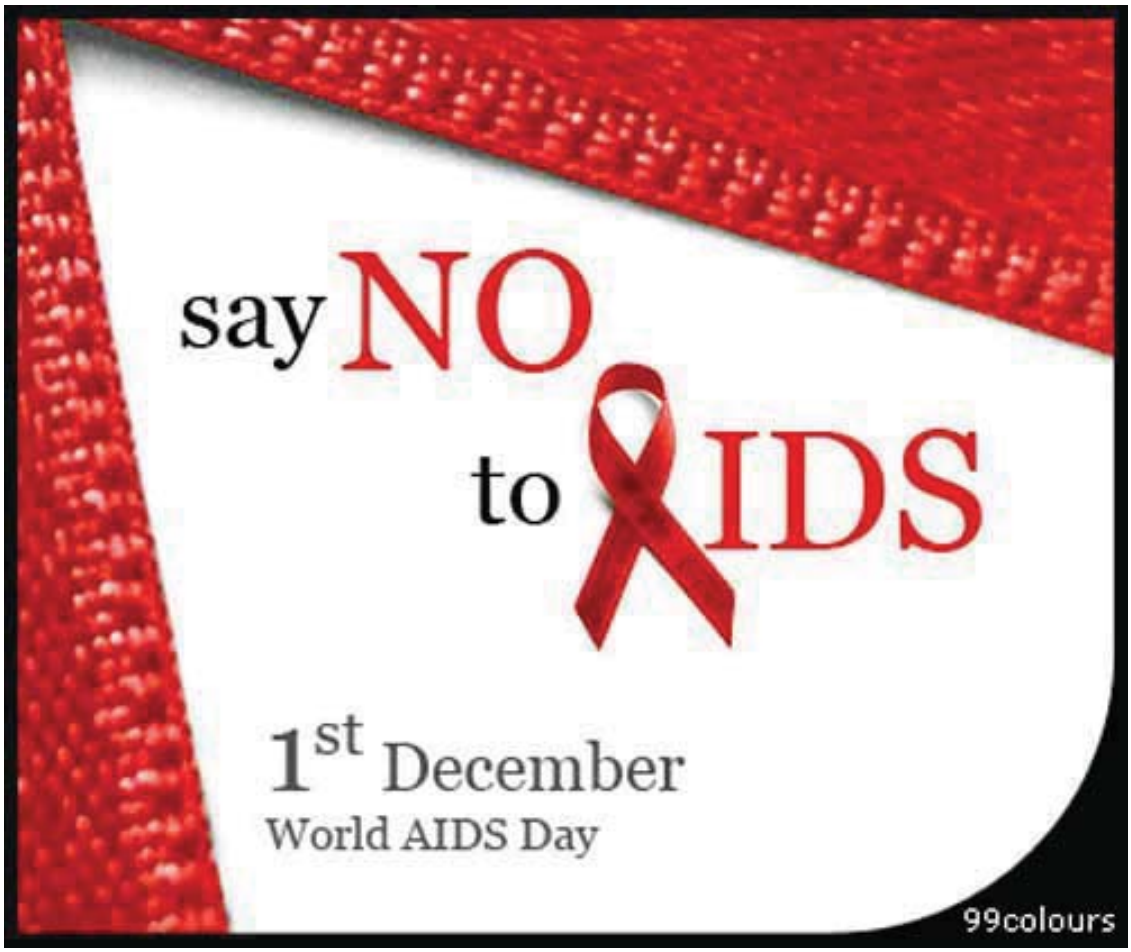
ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2012; December Vol. 69 (No. 12): p. 1023-1120



YU ISSN 0042-8450 vol. 69, br. 12, 2012.

VOJNOSANITETSKI PREGLED

Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine

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Štampa Vojna štamparija, Beograd, Resavska 40 b.

YU ISSN 0042-8450 vol. 69 No. 12, 2012

VOJNOSANITETSKI PREGLED

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

PUBLISHER

Military Health Department, Ministry of Defence, Serbia

Editorial Office: Military Medical Academy, INI; Crnotravska 17, PO Box 33–55, 11040 Belgrade, Serbia. Phone: Editor-in-chief +381 11 3609 311; Main Journal Manager +381 11 3609 479; Fax: +381–11–2669–689; E-mail: vsp@vma.mod.gov.rs
Papers published in the Vojnosanitetski pregled are indexed in: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, Index Medicus (Medline), Excerpta Medica (EMBASE), EBSCO, Biomedicina Serbica. Contents are published in Giornale di Medicine Militare and Revista de Medicina Militara. Reviews of original papers and abstracts of contents are published in International Review of the Armed Forces Medical Services.
The Journal is published monthly. Subscription: Account in Uprava za javna plaćanja in Belgrade. Giro Account No. 840-941621-02 – VMA (za Vojnosanitetski pregled), refer to number 1962-1. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 4,000.00 Din, institutions 8,000.00 Din in Serbia, and foreign subscribers 150 €.

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

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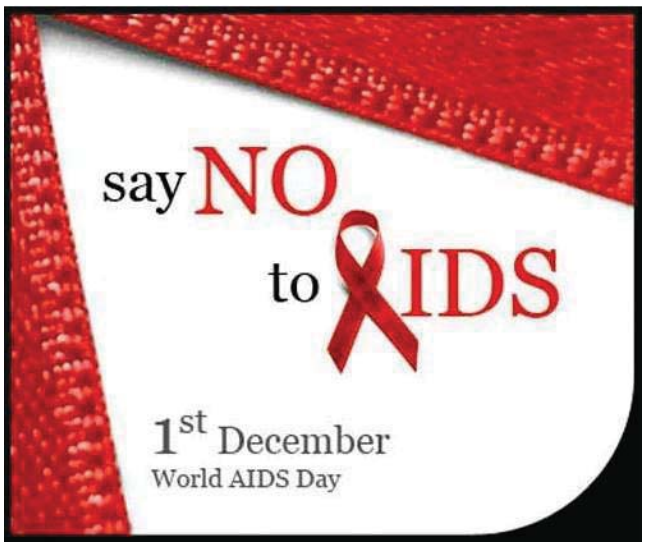

Od 1988. godine, 1. decembra obeležava se Svetski dan borbe protiv AIDS-a sa ciljem podizanja svesti o neophodnosti zajedničkog delovanja svih zemalja sveta u programima prevencije i lečenja ove bolesti. Između 2011. i 2015. godine širom sveta sprovodiće se akcije pod geslom: "*Getting to zero*: *Zero new* HIV *infections*. *Zero discrimination*. *Zero* AIDS *related deaths*".
Uredništvo „Vojnosanitetskog pregleda" poziva sve autore, saradnike i čitaoce časopisa da se aktivno uključe u sprovođenje ovih aktivnosti.

Since 1988 World AIDS Day has been marked on December 1st in order to raise awareness about joint action necessity around the world in HIV/AIDS prevention and treatment programmes. Between 2011 and 2015, World AIDS Day will have the theme of "Getting to zero: Zero new HIV infections. Zero discrimination. Zero AIDS related deaths".
Editorial board of the "Vojnosanitetskog pregleda" invites all authors, readers and collaborators of the Journal to take an active part in these activities.

Poštovani autori, urednici, recenzenti i saradnici Vojnosanitetskog pregleda,

Opraštajući se od stare godine, zahvaljujem vam na izuzetnoj saradnji i podršci uz želje da nam nastupajuća 2013. godina donese još više uspeha i radosti!

SREĆNA NOVA GODINA I BOŽIĆNI PRAZNICI!

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

Dear Authors, Editors, Reviewers, and Collaborators of the *Vojnosanitetski Pregled*,

Saying farewell to 2012, I express my deep gratitute to your extraordinary cooperation and support along with my best whishes that the coming New Year 2013 bring us more success and happiness!

MARRY CHRISTMAS AND A HAPPY NEW YEAR!

Cordially,
Prof. Dr. Silva Dobrić
Editor-in-Chief

EDITORIAL/UVODNIK

Authorship misusing in scientific publications

Zloupotreba autorstva u naučnim publikacijama

Silva Dobrić

Institute for Scientific Information, Military Medical Academy, Belgrade, Serbia

Research results publishing is "the end that adorns an act" of every scientific research, because only published results can influence the development of the applicable scientific field. Due to this, the authors of the published articles are especially appreciated in scientific and professional associations. Most often, it is also the basis for the assessment of scientific contribution of individual researcher and used for his/her advancement in the academic and scientific community by providing him/her a better social and financial status in this way. Therefore, researchers tend to publish more papers, preferably in journals indexed in the renowned databases of scientific publications since the status of the scientific journals is an indirect indicator of the value of articles published in them, and consequently the authors of these articles.

To achieve this goal, some authors resort to a variety of abuses. About plagiarism (download complete or parts of other people's work and their publication under his/her own name) and selfplagiarism (multiple publication of the same or slightly altered his/her own article) as a way of authorship misuse or abuse and ways for their detection and prevention has been already written in our Journal [1–4]. However, by far the most common authorship misuse refers to the so-called undeserved or gift authorship, i.e. including in the byline persons which do not patipcipates in the research realization. This phenomenon is especially pronounced in scientific publications in the field of biomedicine that, very often, especially in recent times, have a growing number of authors. Sometimes, it can be justified, for example, in the case of publishing the results of large clinical multicenter, and, often, multinational studies, but usually this is not the case. For example, in biomedical journals, such is the "Vojnosanitetski pregled" (VSP), articles befalling to the category Case report, often have more than six authors who, judging by their affiliations, are apparently unrelated to their topic.

Undeserved authorship is not, unfortunately, the exclusivity of the so-called "small" scientific communities, such as

Publikovanje rezultata istraživanja predstavlja „konac koji delo krasi" svakog naučnog i stručnog rada jer samo onda kada su objavljeni oni mogu da utiču na razvoj naučne oblasti na koju se odnose. Zbog toga se autorstvo objavljenih radova posebno ceni u naučnim i stručnim krugovima. Najčešće, ono je i osnov za procenu naučnog doprinosa pojedinog istraživača i služi za njegovo napredovanje u akademskoj i naučnoj zajednici obezbeđujući mu, na taj način, bolji društveni i materijalni status. Zbog toga naučni i akademski radnici nastoje objaviti što više radova, po mogućnosti, u časopisima indeksiranim u poznatim bazama naučne publicistike jer status naučnog časopisa predstavlja indirektan pokazatelj i vrednosti radova objavljenih u njima, a time i autora tih radova.

U postizanju ovog cilja, pojedini autori pribegavaju različitim zloupotrebama. O plagijarizmu (preuzimanje celog ili delova tuđeg rada i njihovo publikovanje pod svojim imenom) i autoplagijarizmu (višestruko publikovanje istog ili neznatno izmenjenog sopstvenog rada) kao načinu zloupotrebe autorstva i načinima za njihovu detekciju i sprečavanje već je pisano na stranicama ovog časopisa [1–4]. Međutim, daleko najčešći način zloupotrebe autorstva odnosi se na tzv. nezasluženo ili poklonjeno autorstvo (engl. *undeserved* ili *gift authorship*), tj. dopisivanje imena pojedinog istraživača u radovima u čijoj realizaciji nije ni učestvovao. Ova pojava posebno je izražena u naučnim publikacijama iz oblasti biomedicine koji, vrlo često, pogotovo u poslednje vreme, imaju sve veći broj autora. Ponekad je to i opravdano, npr. u slučaju objavljivanja rezultata velikih kliničkih multicentričnih, neretko i multinacionalnih studija, ali najčešće to nije slučaj, pa tako i na radovima iz kategorije Prikaz slučaja, često bude navedeno i više od šest autora koji, kada se pogledaju njihove afilijacije, očito nemaju nikakve veze sa temom rada.

Nezasluženo autorstvo nije, nažalost, eksluzivitet samo „malih" naučnih sredina, poput naše, već je, sudeći po napisima u časopisima iz različitih naučnih oblasti, rašireno širom

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ours, but, according to articles in journals in different scientific fields, is spread around the world [5–7]. Most often it is justified by friendy and collegial reasons, although, basically, the main reason for this is the common interest of the author and coauthors ("I'll add your name and you'll add mine"), followed by family reasons (a common occurrence among the authors of the same last name), but there are cases of assigning authorship to supervisors or persons who are expected some help, sometimes without their knowledge, in order to achieve certain benefits. However, there are cases of involuntary adding an author, when the author, mostly junior researcher, does it so due to the insistence of his mentor or leading senior researcher, fearing of adverse consequences for his/her status and even denial of authorship if not doing so [7, 8].

For a long time in the academic and scientific community, the ways to prevent the appearance of undeserved authorship have been diseussed. The International Committee of Medical Journal Editors (ICMJE), also known as the Vancouver Group, made a guideline for preparation of manuscripts submitted to biomedical journals in which, among other things, there are the criteria that has to be fulfilled by a researcher to be included among the authors of scientific article [9]. According to this document the authoriship belongs only to those who significantly contribute to all phases of research and preparation of a manuscript for publication:"1) substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the aricle or revising it critically for important intellectual content; 3) final approval to the version to be published". Other collaborators in the research who do not fulfill these criteria are usually mentioned in the Acknowledgements section. The exception are some studies which require a specific profile of professionals (e.g, biostatistician, epidemiologist and pathologist) who, though they do not participate in all the three phases of a scientific work realization, can be listed as authors, if it is estimated that their engagement in the study is not a part of their routine engagement.

In an effort to raise as much a prestige of the VSP and harmonize its editing into line with international standards for biomedical journals the Editorial Board of the Journal, from the beginning of the next year, will demand that all authors (if there are two or more) of the manuscripts submitted to the VSP Editorial Office, to sign a statement of his/her contribution to the work (Authorship statement) that will be published at the end of the article under the heading "Author contributions". We hope it will be the way to reduce the number of "false" authors and that only the names of those who, through their creative, intellectual efforts actually contribute to the realization of scientific work will be listed in the byline.

Another novelty refers to the requirement to sign the statement by each author declaring any potential conflict of interest (Statement of conflicts of interest), which will also, starting from January 2013, be a part of the documentation required in submitting manuscripts to the VSP Editorial Office. This statement is very important for assessing the reliability of the results presented in the paper, if it is found that among the authors there are those who receive honoraria, paid trips to professional meetings, apparatus or reagents

sveta [5–7]. Najčešće se ono opravdava drugarskim i kolegijalnim razlozima, premda se, u osnovi, radi o zajedničkim interesima autora i koatura ("ja ću dopisati tebe, a ti mene"), zatim rodbinskim razlozima (česta je pojava istih prezimena među autorima), a ima slučajeva stavljanja na rad nadređenih ili osoba od kojih se očekuje neka pomoć, ponekad i bez njihovog znanja, sa ciljem ostvarenja određenih beneficija. Međutim, postoje i slučajevi nedobrovoljnog dopisivanja koautora, kada autor, najčešće mlađi istraživač, to čini na insistiranje svog mentora ili rukovodiovca projekta, bojeći se neželjenih posledica po svoj status, pa čak i oduzimanja autorstva, ukoliko to ne učini [7, 8].

Već duže vreme u akademskim i naučnim krugovima vode se rasprave o načinima sprečavanja pojave nezasluženog autorstva. Internacionalni komitet urednika medicinskih časopisa (*International Committee of Medical Journal Editors* – ICMJE), poznat i kao Vankuverska grupa, izradio je svojevrsni vodič za pripremu rukopisa koji se podnose redakcijama biomedicinskih časopisa u kome je, između ostalog, precizirano koje kriterijume mora da zadovolji istraživač da bi mogao biti uvršten među autore naučnog i stručnog članka [9]. Prema ovom dokumentu autorstvo rada pripada samo onima koji su značajno doprineli u svim fazama tokom istraživanja i pripreme rada za objavljivanje: 1) izradi koncepta i dizajna eksperimenta, prikupljanju podataka, odnosno njihovoj analizi i interpretaciji; 2) pisanju prve verzije članka ili kritičkoj reviziji najvažnijih delova rukopisa; 3) davanju saglasnosti na završnu verziju rada koja će se predati za publikovanje. Ostali saradnici na radu koji ne ispunjivaju sve gorenavedene kriterijume obično se navode u sekciji Zahvalnost (engl. *Acknowlegdgment*). Izuzetak predstavljaju neke studije za koje je neophodan određeni profil stručnjaka (npr. biostatističar, patolog ili epidemiolog) koji, iako nisu učestvovali u sve tri faze u relizaciji jednog naučnog rada, mogu biti navedeni kao autori, ako se proceni da njihovo angažovanje u istraživanju nije deo njihovog rutinskog rada.

U nastojanjima da što više podigne ugled časopisa i njegovo uređivanje uskladi sa međunarodnim standardima za biomedicinske časopise Uredništvo „Vojnosanitetskog pregleda" od početka naredne godine zahtevaće da svi autori (ukoliko ih je dva ili više) radova dostavljenih redakciji časopisa potpišu izjavu o svom doprinosu radu (*Authorship statement*) koja će biti objavljena na kraju članka u rubrici „Doprinos autora" (*Author contributions*). Nadamo se da će na taj način broj „lažnih" autora biti smanjen i da će samo imena onih koji su svojim kreativnim, intelektualnim naporima stvarno doprineli realizaciji naučnog rada biti navedena u spisku autora.

Druga novost odnosi se na zahtev za potpisivanje izjave o postojanju ili ne konflikta interesa (*Statement of conflicts of interest*) koji će, takođe, od januara 2013. godine biti obavezan deo dokumentacije prilikom podnošenja rukopisa u redakciju našeg časopisa. Tu izjavu potpisuju svi autori i ona je veoma važna za ocenu pouzdanosti rezultata prezentovanih u radu, ukoliko se ustanovi da među autorima ima onih koji su dobijali honorare, plaćena putovanja na stručne skupove, aparate ili reagense od određenih firmi.

gifts from certain companies.

Deserved authorship and transparency of data on the sources of funding research are definitely a *conditio sine qua non* of ethics in publishing. Therefore, we believe that the introduction of this novelties in the editorial policy of the VSP will contribute to the efforts to establish the right values in scientific publiching and science, in general.

Zasluženo autorstvo i transparentnost podataka o izvorima finansiranja istraživača svakako predstavljaju *conditio sine qua non* etike u publikovanju. Zbog toga verujemo da ćemo uvođenjem ovih novina u uređivačkoj politici "Vojnosanitetskog pregleda" doprineti naporima za uspostavljanje pravih vrednosti u naučnoj publicistici i nauci, uopšte.

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ORIGINALNI ČLANCI

UDC: 618.19-006-036/-037
DOI: 10.2298/VSP1212031I

Ekspresija citokeratina 5/6 i citokeratina 17 u invazivnom karcinomu dojke

Expression of cytokeratins 5/6 and cytokeratin 17 in invasive breast carcinoma

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Apstrakt

Uvod/Cilj. Citokeratini (CK) 5/6 i 17 predstavljaju markere koji se eksprimiraju u bazalnim/mioepitelnim ćelijama dojke, kao i u pojedinim karcinomima dojke. U više studija dokazano je da je ekspresija ovih „bazalnih" markera povezana sa specifičnim histomorfološkim i imunobiološkim karakteristikama tumora, lošom prognozom i karakterističnim genskim zapisom. Cilj našeg rada bio je da se odredi učestalost ekspresije bazalnih citokeratina CK5/6 i CK17 u ispitivanom uzorku duktalnih invazivnih karcinoma dojke, kao i da se ispita postojanje povezanosti između tumorske ekspresije bazalnih citokeratina i kliničko-patoloških prognostičkih faktora: životnog doba, histološkog stepena diferentovanosti, hormonskog receptornog statusa, kao i HER2 (*human epidermal prowth factor receptor 2*) proteinske ekspresije i HER2 genske ampflikacije u tumorskom tkivu. **Metode.** Istraživanje je bilo sprovedeno na uzorku od 121 karcinoma dojke duktalnog, nespecifičnog tipa. Tumorska ekspresija CK5/6 i CK17 utvrđivana je imunohistohemijskom metodom. U radu je ispitivana učestalost pozitivne imunohistohemijske ekspresije CK5/6 i CK17 u karcinomu dojke, kao i povezanost ekspresije bazalnih citokeratina (*basal-like* fenotipa) sa kliničkopatološkim parametrima. **Rezultati.** Pozitivna ekspresija CK5/6 ustanovljena je kod 22%, a CK17 kod 30% ispitivanih uzoraka karcinoma dojke. Tumori sa pozitivnom ekspresijom CK5/6, kao i CK17, karakterisali su se negativnim nalazom receptora za estrogene i progesteron i negativnim HER2 statusom (triplnegativnim fenotipom). Ekspresija bazalnih citokeratina bila je statistički značajno povezana sa lošim stepenom histološke diferencijacije ispitivanih tumora. **Zaključak.** Određivanje ekspresije citokeratina 5/6 i 17 u tumorskom tkivu, samih ili u kombinaciji sa drugim markerima bazalne diferencijacije, može imati klinički značaj u selekciji bolesnica sa agresivnim tipom karcinoma dojke koje zahtevaju primenu posebnih terapijskih modaliteta.

Ključne reči:
dojka, neoplazme; keratin; receptori, estrogenski; receptori, progesteronski; geni, ekspresija; imunohistohemija; prognoza.

Abstract

Background/Aim. Cytokeratins (CK) 5/6 and 17, myoepithelial markers, are also expressed in a proportion of breast carcinomas. Breast carcinomas expressing basal epithelium cytokeratins constitute a tumor subgroup that shows common but heterogeneous morphological, genetical, and immunophenotypical features and is associated with poor clinical outcome. The aim of this study was to determine the incidence of basal expression of cytokines CK5/6 and CK17 in the tested samples of ductal invasive breast cancers, as well as to test the presence of a correlation of tumor expression of basal cytokines and clinicopathological prognostic factors: age, the level of histological differentiation, hormone receptor status, HER2 (*human epidermal prowth factor receptor 2*) protein expresion and HER2 gene amplification in tumorous tissue. **Methods.** Immunohistochemistry (IHC) was used to evaluate the CK5/6 and CK17 status of 121 ductal invasive breast cancers. The results thus obtained were compared with clinicopathological characteristics. **Results.** From the 117 analyzed tumor specimens, 22% and 30% were immunohistochemically positive for CK5/6 and CK17, respectively. Basal cytokeratins showed significant inverse relationship with estrogen and progesterone receptor status and HER2 protein expression. CK5/6 and CK17 immunoreactivities were directly associated with triple-negative phenotype and higher histological grade. **Conclusion.** Our findings are similar to reports that tumours expression of basal cytokeratins are correlated with adverse pathological parameters. Given the limited number of emerging therapeutic targets in these tumors, routine IHC identification of basal-like subtype as a poor prognostic group of breast cancer could be based on the expression of basal CKs.

Key words:
breast neoplasms; keratins; receptors, estrogen; receptors, progesterone; gene expression; immunohistochemistry; prognosis.

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Uvod

Citokeratini (CK) su proteini koji oslikavaju tip epitelnih ćelija, tkivni rast i diferencijaciju, kao i funkcionalno stanje tkiva. Do sada je opisano dvadesetak različitih keratin gena, lokalizovanih na hromozomima 12 ili 17. Svaki od ovih gena enkodira specifičnu keratinsku podjedinicu na osnovu koje se može izvršiti klasifikacija na nisko- i visokomolekularne citokeratine, kao i kisele, odnosno bazne oblike na osnovu njihovih izoelektričnih tačaka [1].

Tokom razvoja i diferencijacije tkiva i organa, eksprimiraju se različite izoforme citokeratina, a različite vrste epitela karakterišu se različitim oblicima citokeratina. Stratifikovani skvamozni epitel pokazuje ekspresiju CK1-6 i 9-17, dok su CK7, 8, i 18–20 identifikovani u jednostavnim, žlezdanim epitelima [2, 3]. Dokazano je da se ekspresija citokeratina tokom onkogeneze ne menja. Većina karcinoma dojke eksprimira CK7, CK8/18 i CK19, diferentovani glandularni fenotip najčešće udružen sa ER pozitivnošću. Nasuprot ovome, značajno manji broj karcinoma dojke eksprimira citokeratine visoke molekularne težine: CK5/6, CK14 i CK17 [2, 4]. Na osnovu ekspresije citokeratina, karcinomi dojke mogu se podeliti u četiri grupe: najučestaliji su tumori koji eksprimiraju isključivo luminalne citokeratine, ređi su mešoviti bazalno/luminalni, dok su čisto bazalni i nulti, oni koji ne esprimiraju citokeratine, retki [4].

Iako je ispitivanje ekspresije različitih citokeratina u karcinomima dojke predmet intenzivnih istraživanja već četvrt veka, skorašnja supklasifikacija karcinoma dojke zasnovana na DNA *microarray* (mikročip) profilu genske ekspresije (DNA čip, genski čip), u centar interesovanja postavlja bazalne karcinome, tumore koji eksprimiraju bazalne citokeratine. Perou i sar. [5] prvi su pokazali da se fenotipska raznolikost karcinoma dojke ogleda u odgovarajućim sistemskim varijacijama genske ekspresije, dok su Sorlie i sar. [6] dokazali da se pet zasebnih tipova karcinoma dojke definisanih tehnologijom *microarray* [luminalni A, luminalni B, sličan normalnoj dojci, HER2 (humai epidermal growth factor receptor 2) pozitivan i bazaloidni tip], karakterišu različitom prognozom bolesti. Bazalni karcinomi dojke, definisani na osnovu genske ekspresije analizom *microarray*, čine do 15% od svih karcinoma dojke [5, 6]. Ova podgrupa nazvana je tako jer neoplastične ćelije bazalnog karcinoma eksprimiraju gene koji se uobičajeno nalaze u normalnim bazalnim/mioepitelnim ćelijama dojke, uključujući one odgovorne za citokeratine visoke molekularne težine, HMW (*high molecular weight*) ili 'bazalne' citokeratine (CK5/6, CK14 i CK17), kao i neke druge markere bazalne diferencijacije [7–9]. Iako poreklo i patogeneza bazalnih karcinoma nije u potpunosti jasna, smatra se da ovi tumori verovatno potiču od CK5 pozitivnih epitelnih progenitornih ćelija dojke. U fiziološkim uslovima, ove ćelije su, preko intermedijalne faze, sposobne da se diferenciraju u pravcu luminalnog epitela (CK8, 18+) i mioepitela (eksprimira glatkomišićni aktin) [2, 4]. U više studija dokazano je da su bazalni karcinomi dojke pretežno loše diferentovani tumori, često sa medularnim i metaplastičnim elementima. Imunohistohemijski, oni su pretežno receptori za estrogen – (ER), receptori za progesteron – (PR) i receptori HER2 – (HER2)-negativni. Pozitivna ekspresija bazalnih markera, dokazano je, povezana je sa lošom prognozom sporadičnih tumora i sa BRCA-1 mutacijama, najverovatnije kao odraz visokog proliferativnog kapaciteta ovih tumora, kao i nepostojanja adekvatne, individualizovane, direktno ciljane terapije [7, 8].

Cilj našeg rada bio je da se odredi učestalost ekspresije bazalnih citokeratina CK5/6 i CK17 u ispitivanom uzorku duktalnih invazivnih karcinoma dojke, kao i da se ispita postojanje povezanosti između tumorske ekspresije bazalnih citokeratina i kliničko-patoloških prognostičkih faktora: životnog doba, histološkog stepena diferentovanosti, hormonskog receptornog statusa, kao i HER2 proteinske ekspresije i HER2 genske ampflikacije u tumorskom tkivu.

Metode

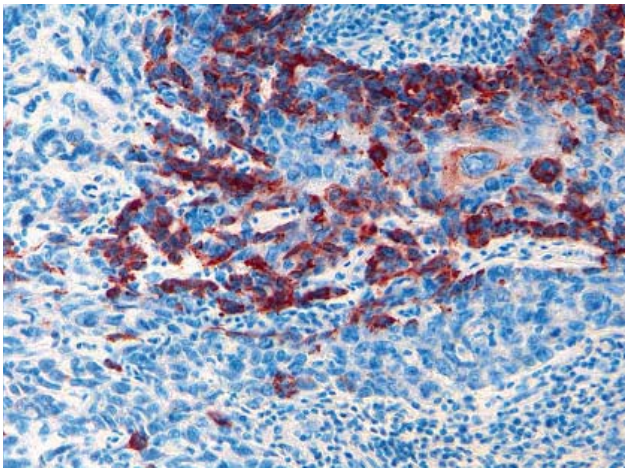
Istraživanje je bilo sprovedeno na uzorku od 121 invazivnog karcinoma dojke nespecifičnog, duktalnog tipa kod bolesnica koje su bile u stadijumu bolesti II A (T1 N1 i T2 N0) (prema Tumor, Nodus, Metastaza – TNM klasifikaciji), operisane u Klinici za operativnu onkologiju Instituta za onkologiju Vojvodine, u periodu od 2000. do 2002. godine. Svi tkivni uzorci bili su fiksirani u 10% formalninu. Stadijum tumorske bolesti bio je određen na osnovu *p* (patološke) TNM klasifikacije karcinoma dojke [10]. Za utvrđivanje stepena histološke diferentovanosti korišćen je semikvantitativni Bloom-Richardson sistem gradiranja modifikovan od strane Symmerb i sar. [11].

Svi tkivni uzorci fiksirani su u formalinu i ukalupljeni u parafin i sečeni su na rezove debljine 5 mikrometara, a zatim nanošeni na pločice i sušeni. Primenjena su sledeća primarna monoklonska antitela u optimalnim ili komercijalnim koncentracijama: ER alfa (1D5 M7047 DAKO), PR (1A6 M3529 DAKO), HER2 /c-erbB-2 protein (A0485 DAKO), citokeratin 5/6 (M7237 DAKO), citokeratin 17 (M7046 DAKO). Imunohistohemijska identifikacija ispitivanih antigena vršena je primenom tehnike streptavidin-biotin-peroksidaza [12]. U negativnm kontrolama nije vršena inkubacija primarnim antitelom. Kao pozitivna kontrola služilo je tumorski neizmenjeno tkivo dojke kao i odgovarajući, unapred izabrani tkivni uzorci.

Kompletno ispitivanje sprovedeno je na uzorku koji je činilo 117 slučajeva.

Ekspresija ER i PR procenjivana je semikvantitativno prema procentu tumorskih ćelija čija su jedra bila jasno ili intenzivno obojena crvenkastosmeđe, pri čemu su kao pozitivni slučajevi vrednovani tumori sa najmanje 10% pozitivnih ćelija [13]. U proceni ekspresije HER2 proteina korišćen je skoring sistem sa četiri kategorije (0–3), na osnovu kog su tumori svrstavani u negativnu (skorovi 0 i 1+), nedefinisanu kategoriju (skor 2+) i pozitivnu (skor 3+), sa kompletnom membranskom imunoreaktivnošću jakog intenziteta u najmanje 30% tumorskih ćelija [13, 14]. Svi slučajevi sa skorom 2+ retestirani su metodom hromogene *in situ* hibridizacije (CISH) u cilju utvrđivanja postojanja genske amplifikacije korišćenjem sistema Zymed SpoT-Light®HER2 CISH™Kit. Uzorci su analizirani svetlosnim mikroskopom na ×40 uvećanju i interpretirani prema priloženim preporukama proizvođača (Zymed® HER2 CISH™ Test Interpretation Guide) [15].

Kao pozitivni citokeratin 5/6 i 17 slučajevi definisani su oni u kojima je ustanovljena makar jedna jasno pozitivna tumorska ćelija. Kao pozitivna unutrašnja kontrola korišćene su obojene bazalne/mioepitelne ćelije duktusa u tumorski neizmenjenom tkivu dojke (Slika 1).



Sl. 1 – Citokeratin 5/6-pozitivna imunohistohemijska reakcija u tumorskim ćelijama (×400)

Povezanost između ekspresije CK5/6, CK17 u tumoru i različitih kliničkopatoloških parametara utvrđivana je χ^2 testom. Razlika na nivou $p < 0.05$ smatrana je statistički značajnom.

Rezultati

Kliničkopatološke karakteristike u ispitivanom uzorku prikazane su u tabeli 1.

Tabela 1
Kliničkopatološke karakteristike ispitivanog uzorka karcinoma dojke

Parametri	n (%)
Životno doba	
< 50 godina	35 (29,91)
≥ 50 godina	82 (70,09)
Histološki gradus	
HG 1	21 (17,95)
HG 2	71 (60,68)
HG3	25 (21,37)
Receptori za estrogen (ER)	
ER negativni	29 (24,79)
ER pozitivni	88 (75,21)
Receptori za progesteron (PR)	
PR negativni	26 (22,22)
PR pozitivni	91 (77,88)
HER2	
negativan	103 (88,04)
pozitivan	14 (11,96)

HER2 – *human epidermal growth factor receptor 2*

Posmatrajući kombinovano vrednosti ekspresije hormonskih receptora i HER2, najviše je bilo nalaza sa pozitivnim hormonskim receptorima uz negativan HER2 status (HR+ HER2-, 88%–75,21%). Negativan hormonski receptorni status uz negativan HER2 (HR- HER2-) ustanovljen je kod 16 (13,68%) slučajeva, negativan hormonski receptorni status uz pozitivan HER2 (HR- HER2+) kod 7 (5,98%), a nalaz pozitivnih hormonskih receptora uz istovremeno pozitivan HER2 (HR+ HER2+) kod 6 (5,13%) slučajeva.

U ispitivanom uzorku ustanovljeno je 26 (22,22%) CK5/6 pozitivnih i 35 (29,91%) CK17 pozitivnih tumora.

Tumori pozitivni na CK5/6 karakteristično su pokazivali koekspresiju CK17. Analizirajući povezanost tumorske ekspresije CK5/6 i CK17 ustanovljena je umereno značajna povezanost ($p = 0,000$ χ^2-testa, a $\chi^2 = 0,564$, sa intervalom poverenja 0,484; 0,645 koji ne sadrži nulu).

Tumori pozitivni na CK5/6 bili su karakteristično ER i PR negativni (tabela 2). Kako je $p = 0,001$ χ^2-testa, a $\chi^2 = 0,298$ sa intervalom poverenja 0,127; 0,469, koji ne sadrži nulu, može se reći da je ustanovljena niska povezanost između ekspresije citokeratina 5/6 i ekspresije ER. Analizirajući povezanost tumorske ekspresije CK5/6 i ekspresije PR ustanovljena je, takođe, značajna povezanost niskog stepena ($p = 0,005$ χ^2-testa, a $\chi^2 = 0,250$ sa intervalom poverenja 0,068; 0,433 koji ne sadrži nulu). χ^2-testom nije utvrđena povezanost između tumorske ekspresije citokeratina 5/6 i HER2 statusa.

Tabela 2
Brojčana (n) i procentualna (%) zastupljenost vrednosti tumorske ekspresije ER, PR i HER2 u odnosu na ekspresiju citokeratina 5/6

Ekspresija citokeratina (CK)	Tumorska ekspresija			
	n	%	n	%
	ER negativni		ER pozitivni	
CK5/6 negativni	16	17,6	75	82,4*
CK5/6 pozitivni	13	50,0*	13	50,0
	PR negativni		PR pozitivni	
CK5/6 negativni	15	16,5	76	83,5*
CK5/6 pozitivni	11	42,3*	15	57,7
	HER2 negativni		HER2 pozitivni	
CK5/6 negativni	78	85,7	13	14,3
CK5/6 pozitivni	24	92,3	2	7,7

$p = 0,005$ (χ^2-test); ER – receptori za estrogen; PR – receptori za progesteron; HER2 – *human epidermal growth factor receptor 2

Slični nalazi dobijeni su i analizom povezanosti ekspresije CK17 sa ER, odnosno PR i HER2 ekspresijom (tabela 3). Tumori pozitivni na CK17 karakteristično su bili ER negativni i PR negativni. U ispitivanom uzorku ustanovljena je značajna povezanost niskog stepena između pozitivne ekspresije CK17 i negativnog nalaza ER, kao i PR ($p = 0,000$ χ^2-testa; a $\chi^2 = 0,339$ sa intervalom poverenja 0,184; 0,494; $p = 0,000$ χ^2-testa; a $\chi^2 = 0,383$ sa intervalom poverenja 0,238; 0,527 koji ne sadrži nulu). Analizirajući povezanost između tumorske ekspresije citokeratina 17 i HER2 statusa, nije utvrđena statistički značajna povezanost ($p = 0,369$).

Tumori pozitivni na CK5/6 i CK17 karakterisično su bili ER, PR i HER2 negativni (triplnegativnog fenotipa). Između tumorske ekspresije citokeratina 5/6 i kombinovane ekspresije hormonskih receptora i HER2 ustanovljena je umerena povezanost ($p = 0,000$ χ^2-testa, $\chi^2 = 0,413$ sa intervalom poverenja 0,265; 0,561 koji ne sadrži nulu. Slični su nalazi dobijeni analizom povezanosti ekspresije CK17 i

Tabela 3

Brojčana (n) i procentualna (%) zastupljenost vrednosti tumorske ekspresije ER, PR i HER2 u odnosu na ekspresiju citokeratina 17

Ekspresija citokeratina (CK)	Tumorska ekspresija			
	n	%	n	%
	ER negativni		ER pozitivni	
CK17 negativni	12	14,6	70	85,4
CK17 pozitivni	17	48,6*	18	51,4
	PR negativni		PR pozitivni	
CK17 negativni	9	11,0	73	89,0
CK17 pozitivni	17	48,6*	18	51,4
	HER2 negativni		HER2 pozitivni	
CK17 negativni	70	85,4	12	14,6
CK17 pozitivni	32	91,4	3	8,6

p = 0,000; (χ^2-test); ER – receptori za estrogen; PR – receptori za progesteron HER2 – *human epidermal growth factor receptor 2

kombinovanog nalaza hormonskih receptora i HER2 (p = 0,000 χ^2-testa; a χ^2 = 0,456 sa intervalom poverenja 0,347; 0,566), pri čemu je utvrđena umerena povezanost između ispitivanih obeležja (tabela 4).

Tumori sa pozitivnom ekspresijom bazalnih citokeratina 5/6 i 17 karakteristično su bili lošeg stepena diferencijacije (tabela 5). Analizirajući povezanost ekspresije CK5/6, kao i CK17 sa histološkim gradusom tumora ustanovljena je statistički značajna povezanost niskog stepena (p = 0,034 χ^2-testa, a χ^2 = 0,234 sa intervalom poverenja 0,062; 0,406; p = 0,000 χ^2-testa, a χ^2 = 0,381 sa intervalom poverenja 0,270; 0,492 koji ne sadrži nulu).

Diskusija

Razlike u toku i ishodu bolesti kod bolesnica sa karcinomom dojke u istom stadijumu inicirale su traganje za novim, pouzdanijim prognostičkim parametrima, koji bi, pored standardnih, bili značajni u identifikaciji bolesnica sa nepovoljnim tokom i ishodom bolesti. Pretpostavka da su razlike u toku i ishodu bolesti kod žena sa karcinomom dojke istih histomorfoloških karakteristika rezultat molekularnih razlika među tumorima rezultirao je višedecenijskim istraživanjima, u kojima je ispitivano na hiljade pojedinačnih gena, kao i većeg broja gena istovremeno. Ipak, tek je molekularna analiza karcinoma dojke metodom analize globalne genske ekspresije (DNA mikročip) donela revolucionaran napredak na ovom polju. Kao visokosofisticirana metoda, ona polako počinje da nalazi svoju praktičnu primenu u razvijenim zemljama sveta [16–19].

Bazalni karcinomi dojke identifikovani u studijama profilisanja globalne genske ekspresije definisani su na osnovu setova ekspresije nekoliko gena. Međutim, većina ovih gena nema dijagnostička antitela koja mogu da se koriste na uzorcima tkiva ukalupljenim u parafin, što čini kliničku identifikaciju ovih tumora svojevrsnim izazovom. Iz tog razloga, različiti autori koriste različite panele biomarkera kako bi stvorili klinički model za identifikaciju bazalnih karcinoma, primenjiv u svakodnevnom radu. Međutim, optimalni imunohistohemijski profil bazalnih karcinoma još je uvek u fazi istraživanja. Predloženo je više imunohistohemijskih kombinacija, ali postoji malo podataka o njihovoj specifičnosti i

Tabela 4

Brojčana (n) i procentualna (%) zastupljenost kombinovane vrednosti ekspresije hormonskih receptora (HR) i HER2 u odnosu na tumorsku ekspresiju citokeratina 5/6 i citkeratina 17

Ekspresija citokeratina (CK)	Ekspresija hormonskih receptora							
	HR+ HER2-		HR+ HER2+		HR- HER2+		HR- HER2-	
	n	%	n	%	n	%	n	%
CK5/6 negativni	74	81,3*	6	6,6	6	6,6	5	5,5
CK5/6 pozitivni	14	53,8	0	0	1	3,8	11	42,3*
CK17 negativni	69	84,1*	6	7,3	5	6,1	2	2,4
CK17 pozitivni	19	54,3	0	0	2	5,7	14	40,0*

p = 0,000 (χ^2-test); HER2 – *human epidermal growth factor receptor 2

Tabela 5

Brojčana (n) i procentualna (%) zastupljenost vrednosti histološkog gradusa (HG) u odnosu na tumorsku ekspresiju citokeratina 5/6 i citokeratina 17

Ekspresija citokeratina (CK)	Histološki gradus					
	HG1		HG2		HG3	
	n	%	n	%	n	%
CK5/6 negativni	19	20,9	57	62,6	15	16,5
CK5/6 pozitivni	2	7,7	14	53,8	10	38,5*
CK17 negativni	21	25,6*	51	62,2	10	12,2
CK17 pozitivni	0	0	20	57,1	15	42,9†

***p = 0,034; †p = 0,000 (χ^2-test)**

Iako su kod žena mlađih od 50 godina bili učestaliji tumori sa pozitivnom ekspresijom citokeratina 5/6 i 17 u odnosu na grupu žena starijih od 50 godina, nije dokazana statistički značaja povezanost između životnog doba bolesnica i ekspresije bazalnih citokeratina (p = 0,280 i p = 0,265).

senzitivnosti u identifikaciji bazalnog podtipa karcinoma definisanog tehnologijom *microarray* [9]. Nielsen i sar. [16], u studiji na University British Columbia, predložili su panel na osnovu kog se bazalni karcinomi dojke definišu kao ER i HER2 negativni i CK5/6 i EGFR pozitivni. Većina autora

danas smatra da se ovi tumori mogu identifikovati samo na osnovu ekspresije HMW citokeratina [9]. Ovo stanovište Rakha i sar. [20–22] potkrepljuju činjenicom da je prevalenca tumora sa ekpsreijom bazalnih CK usaglašena sa učestalošću bazalnih karcinoma definisanih *microarray* studijama, kao i da je jasna povezanost ekspresije bazalnih CK i loše prognoze, dok istovremeno mnogi markeri povezani sa ekspresijom bazalnih CK nemaju dokazan prognostički značaj. Međutim, definicija bazalnih karcinoma na osnovu ekspresije bazalnih citokeratina sigurno nije kompletna s obzirom na to da određen broj karcinoma koji eksprimiraju bazalne CK istovremeno eksprimiraju hormonske receptore ili HER2, kao i da određen broj bazalnih karcinoma definisanih metodom *microarray* ne pokazuje ekspresiju bazalnih CK. Pored toga, postoji i značajan stepen varijabilnosti u određivanju ekspresije bazalnih citokeratina, vezanih prvenstveno za odabir (pojedinačni ili više markera) i vrstu, kao i veličinu uzorka koji se pregleda s obzirom na varijabilnost i heterogenost bojenja [9, 22, 23].

U literaturi se navodi da učestalost ekspresije bazalnih citokeratina CK5/6, CK14 i CK17 iznosi od 4% do 38% [2, 4, 16, 24–28]. Gusterson i sar. [7], detaljno su analizirali ekspresiju citokeratina u benignim i malignim bolestima dojke. Korišćenjem tri antitela koja prepoznaju CK14 (LLO02, KA1 i EKH4) ovi istraživači su sa malim varijacijama dokazali da se svi karcinomi dojke mogu svrstati u dve osnovne grupe: 38% karcinoma eksprimiralo je citokeratine stratifikovanog epitela (CK5, 14 i/ili 17), a ostatak je eksprimirao samo jednostavne epitelne keratine. Ekspresija bazalnih citokeratina u karcinomima dojke bila je udružena sa lošom prognozom. Slični su i nalazi Abd El-Rehim-a i sar. [4]. U ovoj studiji izvedenoj na uzorku od 1 944 slučaja karcinoma dojke, zastupljenost čisto luminalnog fenotipa iznosila je 71%, dok je istovremeno učestalost čisto bazalnog fenotipa bila 1%. U Istraživanju Ribeiro-Silva i sar. [27], mešoviti luminalnobazalni fenotip (CK5+, CK8/18+) ustanovljen je kod 14% ispitivanih karcinoma dojke, dok je čist bazalni fenotip (CK5) ustanovljen kod 5%. Karcinomi koji nisu eksprimirali ni jedan od citokeratina bili su najređi, sa učestalošću manjom od 3% [29]. U našem ispitivanju ekspresija CK5/6 i CK17 utvrđena je kod 22% i 30% slučajeva, što je u skladu sa podacima iz literature, iako na gornjoj granici spektra navedenih učestalosti. Varijacije u nalazima pozitivne ekspresije markera bazalne diferencijacije koje postoje u literaturi predstavljaju rezultat postojećih razlika u ispitivanim uzorcima, kao i različitog vrednovanja pozitivnih slučajeva, s obzirom na to da nivo graničnih vrednosti za njihovu selekciju u različitim studijama varira i kreće se od fokalne pozitivnosti, preko 1%, 5%, 10% do 20%. Pored navedenog, razlike u učestalosti ekspresije pojedinih markera mogu biti rezultat korišćenja različitih komercijalnih antitela i/ili metoda bojenja. S obzirom na to da je dokazano kako nalazi bojenja bazalnih citokeratina mogu biti veoma hetorogeni i varijabilni, dodatne razlike i kontradiktornosti u određivanju ekspresije bazalnih citokeratina mogle bi biti i rezultat sve češće upotrebe metode tkivnih mikronizova, kojom se ne može steći uvid u celokupan uzorak tkiva [9, 22]. Poredeći vrednosti ekspresije bazalnih citokeratina u studijama izvedenim poslednjih nekoliko godina i onim izvedenim ranije, možda se upravo iz tog razloga zapaža tendencija smanjenja učestalosti tumora sa pozitivnom ekspresijom bazalnih citokeratina.

Analizirajući međusobni odnos ekspresije CK5/6 i 17 naši rezultati pokazuju da je nalaz pozitivne tumorske ekspresije bazalnog markera CK5/6 povezan sa koekspresijom bazalnog citokeratina CK17 i obrnuto. Međutim, u grupi CK17 pozitivnih tumora bilo je 12 (13%) slučajeva sa negativnim CK5/6 nalazom, dok su istovremeno tri (11,5%) slučaja sa pozitivnom CK5/6 ekspresijom bila CK17 negativna. Još od kada je utvrđeno da pojedini karcinomi dojke mogu eksprimirati HMW citokeratine, dokazana je značajna koekspresija citokeratina ove grupe: CK5/6, 14 i 17. Sasa i sar. [28] ispitujući ekspresiju bazalnih citokeratina 5/6, 14 i 17 u triplnegtivnim karcinomima dojke, ustanovili su značajnu pozitivnu povezanost u ekspresiji ovih markera, sa međusobnom podudarnošću nalaza od 80%. Iako se ekspresija CK5 i CK14 nalazi u paru u normalnim tkivima, koekspresija oba markera postoji u manje od 30% tumora, što ukazuje da korišćenje jednog bazalnog citokeratina nije dovoljno u identifikaciji bazalnih karcinoma ili tumora agresivnog klničkog ponašanja, što je potvrđeno i u našoj studiji [29].

Prosečna starost bolesnica sa bazalnim karcinomom u različitim studijama varira od 47 do 55 godina. One su obično mlađe i premenopauzalne u odnosu na nebazalne tumore [9, 30]. Analizirajući povezanost starosti bolesnica sa ekspresijom bazalnih i luminalnih markera u karcinomu dojke, u istraživanju Abd El-Rehim-a i sar. [4] ustanovljena je pozitivna korelacija između ekspresije luminalnih markera i starosti bolesnica i, istovremeno, inverzna korelacija bazalnih markera i godina života. Slični su i rezultati Foulkesa i sar. [31] koji su ustanovili povezanost između ekspresije CK5/6 i mlađeg životnog doba. Rakha i sar. [22] nisu u svom istraživnju utvrdili udruženost ekspresije bazalnih, niti mioepitelnih markera i životnog doba bolesnica. Slični su rezultati istraživanja Potemski i sar. [25], kao i Laakso i sar. [32] koji su ispitivali kliničnopatološke karakteristike CK5/6 ili CK17 i CK14 negativnih i pozitivnih tumora. Iako je u našem uzorku kod bolesnica mlađih od 50 godina bio češće zastupljen pozitivan nalaz tumorske ekspresije citokeratina CK5/6 i CK17 u odnosu na grupu starijih bolesnica, ova razlika nije statistički značajna.

Tumori pozitivni na CK5/6, CK14 i CK-17 detektuju su pretežno među ER i PR negativnim tumorima, lošeg stepena diferencijacije [9, 22]. Rezultati nekoliko studija pokazali su značajnu korelaciju ekspresije bazalnih citokeratina i negativnog ER statusa [2, 4, 22]. Eerola i sar. [33] ustanovili su povezanost ekspresije bazalnih citokeratina (CK5/6, 14 i 17) sa negativnim ER i PR statusom i mlađim životnim dobom. Chen i sar. [34] dokazali su inverznu povezanost između ekspresije bazalnih citokeratina (CK5/6 i 14) i ER u ispitivanom uzorku karcinoma dojke kod žena mlađih od 35 godina. Naši rezultati su u skladu sa podacima iz literature.

Postoje kontroverzni nalazi o povezanosti tumorske ekspresije bazalnih citokeratina i HER2 statusa. Iako je u pojedinim studijama utvrđena povezanost između ekspresije bazalnih markera i HER2 negativnosti, objavljeni su i nalazi povezanosti između bazalnih citokeratina i HER2 povećane ekspresije [22, 24, 32, 34]. Istovremeno, rezultati studija *microar-*

ray dokazali su da su bazalni karcinomi dojke predominantno HER2 negativni. Naši rezultati u skladu su sa navedenim podacima iz literature. Kao i u većini drugih studija, ni u našoj nije nađena povezanost između ovih parametara. Još uvek nema dostupnih podataka o prognozi i predikciji terapeutskog odgovora za bolesnice sa HER2 amplifikovanim tumorima pozitivnim na bazalne CK [24, 32, 34].

U literaturi se nalazi dosta zbunjujućih podataka o međusobnoj podudarnosti bazalnih karcinoma, tumora koji eksprimiraju bazalne citokeratine i triplnegativnih karcinoma. S obzirom na to da su bazalni tumori uglavnom ER i PR negativni i ne pokazuju HER2 ekspresiju, pojedini autori izjednačavaju kategoriju bazalnih karcinoma dojke i triplnegativnih tumora. Međutim, iako bazalni i triplnegativni karcinomi (ER, PR i HER2 negativni tumori) umnogome dele zajedničke karakteristike, uključujući činjenicu da se oba tipa pojavljuju češće kod mlađih bolesnica (< 50 godina), da se često javljaju prema tipu interval karcinoma u zemljama sa populacionom skrining mamografijom, kao i da su značajno agresivniji od tumora drugih molekularnih podgrupa, a da je jedini terapeutski modalitet sistemske terapije hemioterapija, smatra se da triplnegativni karcinomi dojke, ipak, ne predstavljaju zaseban entitet, odnosno da čine šaroliku grupu, te da se iz tog razloga bazalni i triplnegativni karcinomi ne mogu posmatrati kao istovrsna kategorija [9, 30]. U prilog ovim tvrdnjama idu podaci dobijeni analizama *microarray* kojima je dokazano da u bazalnoj grupi tumora 15–54% njih eksprimira ER, PR ili HER2. Ispitujući ekspresiju bazalnih CK i EGFR u triplnegativnim tumorima ustanovljena je njihova ekspresija kod svega 56–84% tumora [30]. Istovremeno, iako je očigledno da su triplnegativni karcinomi dojke većinom bazalnog fenotipa, pažljiva analiza tehnologijom *microarray* profilisane ekspresije pokazala je da triplnegativni karcinomi mogu pripadati i drugim molekularnim podgrupama tumora, kao što je podtip sličan normalnoj dojci. Od značaja je činjenica da podtip karcinoma dojke sličan normalnoj dojci ima nešto bolju prognozu od bazalnih karcinoma dojke, ali ne pokazuje odgovor na neoadjuvantnu hemoterapiju na isti način kao što čine bazalni karcinomi. Triplnegativni karcinomi čine 10–17% od svih karcinoma dojke, zavisno od sistema korišćenog za definisanje ER i PR pozitivnosti, kao i metode određivanja HER2 statusa, i naši nalazi su u skladu sa vrednostima učestalosti ovih tumora koje se navode u literaturi. Rakha i sar. [29] su u uzorku koji je činila serija od 1 944 karcinoma ustanovili triplnegativan fenotip kod 16,3% slučajeva, pri čemu je većina njih predstavljala loše diferentovane duktalne karcinome nespecifičnog tipa. U ovom radu dokazana je pozitivna povezanost triplnegativnog statusa sa većim dimenzijama tumora, potiskujućom tumorskom marginom, razvojem recidiva, udaljenih metastaza i lošim ishodom bolesti. Povezanost je, takođe, ustanovljena i sa gubitkom ekspresije androgenih receptora i E-cadherina, i pozitivnom ekspresijom bazalnih citokeratina, P-cadherina, p53 i EGFR. Bolesnici sa triplnegativnim karcinomom koji istovremeno eksprimiraju bazalni fenotip imaju značajno kraće preživljavanje u odnosu na triplnegativne karcinome koji ne pokazuju ekspresiju bazalnih markera. Ovi autori prepuručuju da se svi triplnegativni karcinomi dojke boje bazalnim CK i androgenima.

U našoj studiji ustanovljeno je da je ekspresija bazalnih citokeratina CK5/6 i 17 značajno povezana sa lošim stepenom histološke diferencijacije što je u skladu sa podacima iz literature, u kojima se navodi da su tumori sa pozitivnim nalazom ekspresije bazalnih citokeratina, ili bazalni karcinomi definisani *microarray* genskom ekspresijom pretežno gradusa [2–4, 20, 22, 25, 35–37].

U literaturi se navodi da se u bazalnim karcinomima broj mitoza kreće od 25/10HPF do čak 40/HPF [38, 39]. Ovi tumori su pretežno oskudne strome, potiskujuće invazivne margine, sa centralnom komedo ili geografskom nekrozom i limfocitnim infiltratom u stromi. Pored toga, mogu se karakterisati prisustvom metaplastičnih elemenata, centralnim ožiljkom i glomeruloidnom mikrovaskularnom proliferacijom [25, 38]. Iako postoji snažna veza između svih navedenih karakteristika i bazalnog tipa karcinoma dojke, što može olakšavati njihovu identifikaciju, njihove morfološke odlike generalno nisu specifične, i pojedine od navedenih karakteristika mogu se videti i u drugim loše diferentovanim tumorima nezavisno od njihovog fenotipa. Iz tog razloga, ističe se značaj imunohistohemijske detekcije specifičnih (bazalnih) markera, kao realne i jednostavne metode kojom bi se ovi tumori (invazivni karcinomi dojke nespecifičnog tipa sa bazalnim fenotipom) mogli identifikovati u svakodevnoj praksi [29]. Istovremeno, nameće se zaključak da se bazalni tumori, medularni karcinomi i karcinomi dojke nastali kod nosioca BRCA1 mutacija (ili BRCA1 disfunkcionalnim putem) u značajnoj meri poklapaju po svojim morfološkim karakteristikama, kao što dele i sličan imunohistohemijski profil. Bazalne karcinome i tumore nastale kod nosioca germinativnih BRCA1 mutacija, dokazano je, karakteriše loša prognoza [6, 24, 31, 33, 40–45]. Genskim klaster analizama tehnologijom *microarray*, Sorlie i sar. [44] dokazali su da familijarni BRCA1 mutirani tumori pripadaju pretežno grupi bazalnih karcinoma.

Zaključak

Određivanje ekspresije bazalnih citokeratina (CK5/6, CK14, CK17) u karcinomima dojke, samih ili u kombinaciji sa drugim markerima bazalne diferencijacije, nalazi svoje mesto u svakodnevnom radu patologa. Na ovaj način omogućava se selekcija bolesnica sa agresivnim tipom karcinoma koje zahtevaju primenu posebnih terapijskih modaliteta. Istovremeno, postavlja se sumnja u postojanje BRCA1 germinativnih mutacija, naročito kada se radi o tumorima negativnog hormonskog receptornog statusa.

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Primljen 30. XII 2010.
Prihvaćen 16. IX 2011.

ORIGINALNI ČLANAK

UDC: 616.314-089.23::616.716.1/.4-007.26
DOI: 10.2298/VSP1212039S

Analiza varijacija sagitalnog položaja viličnih kostiju u malokluziji skeletne klase III

Analysis of variation of sagittal position of the jaw bones in skeletal class III malocclusion

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Apstrakt

Uvod/Cilj. Malokluzija skeletne klase III je nesklad u sagitalnom odnosu vilica, nastao usled neusklađenosti njihove razvijenosti i/ili položaja, što rezultira dominantnim izgledom donje vilice u facijalnom profilu. Cilj ove studije bio je da se utvrde varijacije sagitalnog položaja viličnih kostiju prema kranijalnoj bazi kod ispitanika sa skeletnom klasom III, radi što ranijeg dijagnostikovanja ove malokluzije. **Metode.** Pedesetoro dece i isto toliko odraslih sa skeletnom klasom III, oba pola, pregledano je i selekcionisano na osnovu nalaza ugla sagitalnog međuviličnog odnosa (ANB) ≤ 0° iz kefalometrijske analize profilnih telerendgenskih snimaka glave. Ispitanici su bili grupisani prema uzrastu. Prvu grupu, činila su deca starosti 6–12 godina, a drugu grupu odrasli starosti 18–26 godina. Mereni su uglovi prognatizma gornje vilice (SNA), prognatizma donje vilice (SNB) i ANB. Na osnovu dobijenih rezultata, unutar pripadajuće grupe učinjena je supklasifikacija na podgrupe, između kojih je procenjivana značajnost razlike izmerenih vrednosti. U obe grupe određivana je značajnost korelacije utvrđenih vrednosti. **Rezultati.** Prosečne vrednosti ugla SNA iznosile su kod dece 77,36 ± 3,58° i 77,32 ± 4,88° kod odraslih, a ugla SNB 79,46 ± 3,91° u grupi dece i 81,12 ± 3,76° kod odraslih. Ugao ANB prosečno je iznosio kod dece -2,10 ± 2,07°, a kod odraslih -4,00 ± 2,34°. U obe grupe utvrđena je značajna korelacija izmerenih vrednosti i značajna razlika u vrednostima svih merenih parametara između ispitanika iz različitih podgrupa ($p < 0,01$). **Zaključak.** Najčešća morfološka varijacija sagitalnog položaja gornje vilice je njen retrognatizam, koji je u jednakoj meri zastupljen kod dece i kod odraslih. Sagitalni položaj donje vilice kod najvećeg broja odraslih bio je prognat, dok je kod dece prognatizam donje vilice bio prisutan u manjoj meri.

Ključne reči:
malokluzija, klase III; maksila; mandibula; kefalometrija.

Abstract

Background/Aim. Skeletal Class III malocclusion is a discrepancy in the sagittal jaw relationship, due to imbalances in their development and/or position, resulting in the dominant appearance of the lower jaw in facial profile. The aim of this study was to determine variations in the sagittal position of the jaw bones to the cranial base in subjects with skeletal Class III, for the earliest possible diagnosis of malocclusion. **Methods.** Fifty children and as many adults with skeletal Class III, both sexes, were examined and selected, based on the findings of sagittal interjaw relationship (ANB) ≤ 0° from the cephalometric analysis of tele-x-ray profile head shots. The subjects were grouped according to age. The first group consisted of children aged 6–12 years, and another group, of adults aged 18–26 years. We measured the angles of maxillary prognathism (SNA), mandibular prognathism (SNB) and ANB. Based on these results, within the respective groups subclassification into the subgroups was done, among which a significant difference measured values was evaluated. In both groups a significant correlation of the determined values was evaluated. **Results.** An average SNA angle ranged 77.36 ± 3.58 in children and 77.32 ± 4.88 in adults, while an average SNB angle was 79.46 ± 3.91 in the group of children and 81.12 ± 3.76 in adults. An average ANB angle was -2.10 ± 2.07 in children, and -4.00 ± 2.34 in adults. In both groups, a significant correlation between the measured values and a significant difference in the values of all the measured parameters were found between patients from different subgroups ($p < 0.01$). **Conclusion.** The most common morphological variation of sagittal position of the upper jaw is its retrognatism, which is equally present in both children and adults. Sagittal position of the lower jaw in most of the adults was prognathic, while mandible prognathism in the children was less present.

Key words:
malocclusion, angle class III; maxilla; mandible; cephalometry.

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Uvod

Malokluzija skeletne klase III predstavlja nesklad u sagitalnom (anterioposteriornom) odnosu vilica, nastao usled neusklađenosti njihove razvijenosti i/ili položaja, što rezultira u dominantni izgled donje vilice u facijalnom profilu. Formiranju ove malokluzije najčešće doprinosi sagitalna nerazvijenost i retrognatizam gornje vilice, sagitalna prerazvijenost i prognatizam donje vilice, ili odstupanja u veličini i/ili položaju viličnih kostiju. Osim neuskađenosti sagitalnog međuviličnog odnosa, moguća su odstupanja u njegovoj vertikalnoj i transverzalnoj dimenziji. Uzimajući u obzir ocenu položaja gornje vilice, donje vilice, dentoalveolarnih nastavaka viličnih kostiju, kao i vertikalne komponente razvoja, izračunate su čak 243 kombinacije u malokluziji skeletne klase III [1].

Studije rasta koje je vršio Singh [2] govore nam da je povećanje dužine tela donje vilice u najvećoj meri zabeleženo u uzrastu od 5 do 13 godina. Isti autor nalazi da se razlike u izgledu donje vilice kod klase III i donje vilice kod normalnog sagitalnog međuviličnog odnosa – klase I, odnose na promene u veličini koje daju značajno povećanje obima promena u morfologiji, a koje, opet, utiču na njenu krajnju veličinu. Takođe, on sugeriše da je i kranijalna baza važna za uvećanje dužine i širine donje vilice, kao i da meka tkiva učestvuju u determinisanju i upotpunjuju završno modeliranje izgleda skeletne klase III [3–5]. Oblik donje vilice može se opisivati vrlo rano u toku razvoja, a jednom uspostavljena ontogeneza podložna je samo ograničenoj remodelirajućoj aktivnosti. Morfološke varijacije donje vilice u skeletnoj klasi III, po nekim autorima, mogu biti do te mere naglašene, da se na osnovu njih može načiniti supklasifikacija ove malokluzije [6, 7].

Sa ili bez prognatizma donje vilice, jedna od najčešće prisutnih komponenti u facijalnoj morfologiji bolesnika sa malokluzijom skeletne klase III, jeste nedovoljna razvijenost srednjeg masiva lica, zbog čega druga grupa autora sugeriše da je upravo ta anatomska struktura odlučujući faktor za klasifikaciju ovih bolesnika [2, 8–11]. Studije pokazuju da je u toku razvoja humanih fetusa centralni deo facijalnog kompleksa jasno prepoznatljiv rano, već u 9. nedelji fetalnog života. Neki autori pretpostavljaju da je prenatalni rast facijalnih struktura predominantan u sagitalnoj ravni i da sagitalni položaj gornje vilice zavisi od promena na prednjoj kranijalnoj bazi, što verovatno omogućava uspostavljanje okluzalnih odnosa klase I [2]. Te studije sugerišu da se morfologija maksilarnog kompleksa uspostavlja rano u toku razvoja i da se, po istom modelu, nastavlja postnatalno [12]. Rast gornje vilice u sagitalnoj, vertikalnoj i transverzalnoj ravni odvija se rastom na fronto-maksilarnoj, palatomaksilarnoj i srednjenepčanoj suturi [13]. Stepen suturalnog rasta može varirati, biti insuficijentan i proizvesti prilično velik deficit u anterioposteriornom rastu, što može imati značajan uticaj na razvoj skeletne klase III.

Osim neusklađenosti skeletnih međuviličnih odnosa, u arhitekturi skeletne klase III čest nalaz je i nepravilnost dentalnih odnosa – obrnut preklop sekutića. U nekim slučajevima nicanje gornjih sekutića u obrnutom preklopu može izazvati zastoj u sagitalnom pozicioniranju alveolarnog grebena gornje vilice, tako da se, kao odgovor, javlja rast u pravcu nadole i napred, što predstavlja funkcionalnu prinudu [14]. To nam sugeriše da rana korekcija okluzalnih odnosa klase III može uspostaviti povoljniji kranio-facijalni model skeletnog rasta [12]. Ovakav nalaz, usmerio je naše istraživanje na ispitivanje varijacija u sagitalnom položaju vilica kod ispitanika sa različitim tipovima skeletne klase III, s ciljem što ranijeg dijagnostikovanja ove malokluzije i utvrđivanja optimalnih metoda u njenom lečenju.

Metode

Ispitivanjem su bila obuhvaćena deca i odrasli sa skeletnom klasom III, koji su pregledani u Vojnomedicinskoj akademiji (VMA), Beograd, a koji ranije nisu ortodontski lečeni. U ispitivanje nisu bili uključeni ispitanici sa kongenitalnim anomalijama, rascepima i anodoncijama pojedinih zuba. Svim ispitanicima učinjena je gnatometrijska analiza gipsanih studijskih modela na osnovu anatomskih otisaka vilica. Potom je sprovedena rendgen dijagnostika koja je podrazumevala izradu i analizu ortopantomografskih i profilnih telerendgenskih snimaka glave u njenom prirodnom položaju, po standardnoj proceduri [15]. Na telerendgenskim snimcima određene su relevantne kefalometrijske tačke, prave i uglovi, koji su korišćeni u kefalometrijskim merenjima, (slika 1). Ispitanici su selekcionisani na osnovu vrednosti ugla

Sl. 1 – Kefalometrijske tačke i parametri korišćeni u selekcionisanju grupa i tipova malokluzije III skeletne klase

S – *Sella* – centar turskog sedla; N – *Nasion* - tačka spoja fronto-nazalne i internazalne suture; A – *Subspinale* – granična tačka između tela gornje vilice i gornjeg alveolarnog nastavka; B – *Supramentale* – granična tačka između tela donje vilice i donjeg alveolarnog nastavka; SN – prednja kranijalna baza; NA – linija prognatizma gornje vilice; NB – linija prognatizma donje vilice; SNA – ugao maksilarnog prognatizma, određuje sagitalni položaj gornje vilice prema prednjoj bazi lobanje; SNB – ugao mandibularnog prognatizma, određuje sagitalni položaj donje vilice prema prednjoj bazi lobanje; ANB – ugao sagitalnog međuviličnog odnosa.

sagitalnog međuviličnog odnosa (ANB) ≤ 0°, a zatim grupisani prema uzrastu. Prvu grupu, činilo je pedesetoro dece sa skeletnom klasom III i mešovitom denticijom, oba pola (25 muških i 25 ženskih), uzrasta 6–12 godina. Drugu grupu, činilo je pedesetoro odraslih ispitanika sa skeletnom klasom III, oba pola (28 muških i 22 ženska), starosti 18–26 godina. Nakon toga, svakom ispitaniku, unutar pripadajuće grupe, određen je tip malokluzije skeletne klase III, na osnovu sagitalnog položaja gornje vilice prema kranijalnoj bazi, merenog uglom prognatizma gornje vilice (SNA) i sagitalnog položaja donje vilice prema kranijalnoj bazi, merenog uglom prognatizma donje vilice (SNB). Na taj način, u svakoj grupi izvršena je supklasifikacija na podgrupe:

- I – ortognatizam gornje vilice i prognatizam donje vilice (SNA ⊥ SNB ↑)
- II – retrognatizam gornje vilice i ortognatizam donje vilice (SNA↓ SNB ⊥)
- III – bimaksilarni retrognatizam (SNA↓ SNB↓)
- IV – bimaksilarni prognatizam (SNA↑ SNB ↑)
- V – retrognatizam gornje vilice i prognatizam donje vilice (SNA↓ SNB ↑)
- VI – bimaksilarni ortognatizam (SNA ⊥ SNB ⊥)

jenih vrednosti korišćen je χ^2 test. Za izmerene vrednosti uglova SNA i SNB utvrđivana je značajnost korelacije korišćenjem *Pearson Correlation* testa. Statističko tumačenje u svim analizama prihvatano je na nivou verovatnoće $p \leq 0,05$.

Rezultati

U grupi dece sa skeletnom klasom III, kod najvećeg broja bio je prisutan bimaksilarni retrognatizam (podgrupa III, 40%), a u grupi odraslih kombinacija ortognatizma gornje vilice i prognatizma donje vilice (podgrupa I, 28%) (tabela 1).

Rezultati našeg istraživanja pokazuju da je retrognatizam gornje vilice prisutan u gotovo jednakoj meri kod dece (66%) i kod odraslih (64%) sa skeletnom klasom III (tabela 2), dok je prognatizam donje vilice u većoj meri zastupljen kod odraslih (62%), nego kod dece (40%) (tabela 2). Kombinacija maksilarnog retrognatizma i mandibularnog prognatizma (podgrupa V) bila je zastupljena u grupi dece 14% ispitanika, a kod odraslih kod više od četvrtine (26%). Retrognatizam gornje vilice, bez prognatizma donje vilice (podgrupe II i III) bio je zastupljen kod 52% dece i 38% odraslih.

Tabela 1

Distribucija ispitanika prema tipu malokluzije skeletne klase III

Tip malokluzije	Deca (n = 50)	Odrasli (n = 50)
	n (%)	n (%)
I	8 (16)	14 (28)
II	6 (12)	7 (14)
III	20 (40)	12 (24)
IV	5 (10)	4 (8)
V	7 (14)	13 (26)
VI	4 (9)	

Tabela 2

Distribucija ispitanika sa maksilarnim retrognatizmom i mandibularnim prognatizmom

Morfološka varijacija sagitalnog položaja viličnih kostiju	Deca (n = 50)	Odrasli (n = 50)
	n (%)	n (%)
Maksilarni retrognatizam		
- prisutan	33 (66)	32 (64)
- odsutan	17 (34)	18 (36)
Mandibularni prognatizam		
- prisutan	20 (40)	31 (62)
- odsutan	30 (60)	19 (38)

Vilični ortognatizam podrazumevao je normalne vrednosti ugla sagitalnog položaja vilice prema kranijalnoj bazi (SNA = 80–82°, SNB = 78–80°), retrognatizam, njegove smanjene vrednosti, (SNA < 80°, SNB < 78°), a prognatizam, veće vrednosti ovog ugla (SNA > 82°, SNB > 80°). U grupi dece formirano je svih šest podgrupa, dok u grupi odraslih nije bilo ispitanika sa bimaksilarnim ortognatizmom.

Nakon učinjene klasifikacije na grupe i supklasifikacije na podgrupe unutar svake grupe, upoređeni su dobijeni rezultati merenja ispitivanih parametara SNA, SNB i ANB između ispitanika sa različitim tipovima skeletne klase III, unutar svake grupe. Za utvrđivanje značajnosti razlike dobi-

Prognatizam donje vilice, bez prisustva retrognatizma gornje vilice (podgrupe I i IV), zapažen je kod 26% dece i 36% odraslih. Kod naših ispitanika sa skeletnom klasom III u obe grupe konstatovan je vilični ortognatizam. Ortognat sagitalni položaj gornje vilice (podgrupe I i VI kod dece i podgrupa I kod odraslih), bio je prisutan u nešto manjoj meri kod dece (24%), nego kod odraslih (28%). Ortognat sagitalni položaj donje vilice (podgrupe II i VI kod dece i podgrupa II kod odraslih), bio je prisutan u nešto većoj meri kod dece (20%), nego kod odraslih (14%). U naše istraživanje bili su uključeni i netipični slučajevi skeletne klase III sa prognatizmom gornje vilice, odnosno retrognatizmom donje vilice, pri čemu

je prognatizam gornje vilice konstatovan samo u kombinaciji sa izraženijim prognatizmom donje vilice (podgrupa IV), a retrognatizam donje vilice, samo u kombinaciji sa izraženijim retrognatizam gornje vilice (podgrupa III), (tabela 1).

Vrednosti ugla SNA u grupi 1 kretale su se u intervalu od 70,00° do 84,00°, sa prosečnom vrednošću od 77,36° (tabela 3). Ugao SNA imao je najveću prosečnu vrednost kod ispitanika sa malokluzije tipa IV skeletne klase III, gde je ona iznosila 83,40°, a najmanju vrednost od 74,05°, kod ispitanika sa tipom III ove malokluzijom (tabela 4). Rezultati χ^2-testa pokazali su da između ispitivanih tipova malokluzije skeletne klase III postoji statistički visokoznačajna razlika u prosečnim vrednostima ugla SNA ($p < 0,01$).

minimalnih 70,00° do maksimalnih 90,00° (tabela 3). Njegova najveća prosečna vrednost od 84,20° utvrđena je kod ispitanika iz podgrupe IV, a najmanja od 75,65° u podgrupi III (tabela 4). Vrednosti ovog ugla kod ispitanika sa različitim tipovima malokluzije skeletne klase III, prema rezultatima χ^2-testa, bili su visokoznačajno različite ($p < 0,01$).

U grupi 2, merenjem ugla SNB, utvrđeno je postojanje njegovih vrednosti u opsegu od 70,00° do 91,00°, prosečno 81,12° (tabela 3). Kao i u grupi 1, najveću prosečnu vrednost imali su ispitanici iz podgrupe IV, 88,00°, a najmanju oni iz III podgrupe, 74,25°, što je po rezultatima χ^2-testa, visokoznačajno različito ($p < 0,01$) (tabela 5).

Tabela 3

Vrednosti merenih uglova kod ispitanika sa malokluzijom skeletne klase III

Ispitivani uglovi	Deca (n = 50)				Odrasli (n = 50)			
	min	max	med	$\bar{x} \pm SD$	min	max	med	$\bar{x} \pm SD$
SNA (°)	70,00	84,00	77,00	77,36 ± 3,58	65,00	87,00	77,50	77,32 ± 4,88
SNB (°)	70,00	90,00	80,00	79,46 ± 3,91	70,00	91,00	82,00	81,12 ± 3,76
ANB (°)	-9,00	0,00	-2,00	-2,10 ± 2,07	-10,00	0,00	-4,00	-4,00 ± 2,34

SNA – ugao prognatizma gornje vilice; SNB – ugao prognatizma donje vilice; ANB – ugao sagitalnog međuviličnog odnosa

Tabela 4

Vrednosti merenih uglova kod dece sa različitim tipovima malokluzije skeletne klase III

Ispitivani uglovi	Tip malokluzije skeletne klase III						χ^2	p
	SNA⊥ SNB ↑ podgrupa I (16%)	SNA↓ SNB⊥ podgrupa II (12%)	SNA↓ SNB↓ podgrupa III (40%)	SNA↑ SNB ↑ podgrupa IV (10%)	SNA↓ SNB ↑ podgrupa V (14%)	SNA⊥ SNB ⊥ podgrupa VI (8%)		
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$		
SNA (°)	80,75 ± 0,89	76,67 ± 1,75	74,05 ± 1,93	83,40 ± 0,55	77,71 ± 1,11	80,00 ± 0,00	0,00	< 0,01
SNB (°)	83,63 ± 2,88	79,17 ± 0,98	75,65 ± 2,01	84,20 ± 1,30	82,14 ± 1,22	80,00 ± 0,00	0,00	< 0,01
ANB (°)	-2,88 ± 2,85	-2,00 ± 1,23	-1,60 ± 1,50	-0,80 ± 1,09	-4,43 ± 1,99	0,00 ± 0,00	0,00	< 0,01

⊥ normalna vrednost ugla, ↑ vrednost ugla viša od normalne, ↓ vrednost ugla niža od normalne; SNA – ugao prognatizma gornje vilice; SNB – ugao prognatizma donje vilice; ANB – ugao sagitalnog međuviličnog odnosa

Tabela 5

Vrednosti merenih uglova kod odraslih sa različitim tipovima malokluzije skeletne klase III

Ispitivani uglovi	Tip malokluzije III skeletne klase					χ^2	p
	SNA⊥ SNB ↑ podgrupa I (28%)	SNA↓ SNB⊥ podgrupa II (14%)	SNA↓ SNB↓ podgrupa III (24%)	SNA↑ SNB ↑ podgrupa IV (8%)	SNA↓ SNB ↑ podgrupa V (26%)		
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$		
SNA (°)	80,86 ± 1,29	76,71 ± 1,38	71,25 ± 3,98	85,50 ± 1,29	76,92 ± 2,36	0,00	< 0,01
SNB (°)	84,21 ± 2,23	79,14 ± 1,07	74,25 ± 2,42	88,00 ± 2,16	83,08 ± 1,50	0,00	< 0,01
ANB (°)	-3,64 ± 1,65	-2,43 ± 1,51	-3,00 ± 2,30	-2,50 ± 1,29	-6,15 ± 2,19	0,00	< 0,01

⊥ normalna vrednost ugla, ↑ vrednost ugla viša od normalne, ↓ vrednost ugla niža od normalne; SNA – ugao prognatizma gornje vilice; SNB – ugao prognatizma donje vilice; ANB – ugao sagitalnog međuviličnog odnosa

Kod ispitanika grupe 2, ugao SNA imao je širi interval varijacija i nižu prosečnu vrednost. Njegova vrednost varirala je od 65,00° do 87,00°, a prosečno je iznosila 77,32° (tabela 3). Ispitanici iz podgrupe III ove grupe imali su prosečno najnižu (71,25°) a iz podgrupe IV najvišu vrednost ugla SNA, koja je kod njih iznosila 85,50° (tabela 5). Kako je ustanovljeno χ^2-testom, razlika između ovih vrednosti bila je statistički visokoznačajna ($p < 0,01$).

Ugao SNB, kod grupe 1 ispitanika, imao je prosečnu vrednost 79,46°, a vrednosti su mu se kretale u rasponu od

Merenjem ugla ANB u grupi 1 ispitanika utvrđene su njegove vrednosti u rasponu od -9,00° do 0,00°, prosečno -2,10° (tabela 3). Najnižu vrednost ovaj ugao imao je kod podgrupe V, gde je ona prosečno iznosila -4,43°, a najvišu vrednost od 0,00° u podgrupi VI (tabela 4). Razlika u vrednostima ugla ANB merenim kod različitih tipova malokluzije skeletne klase III, bila je statistički signifikantna ($p < 0,01$).

Ispitanici grupe 2, imali su minimalnu vrednost ugla ANB od -10,00°, a maksimalnu, kao i ispitanici grupe 1, 0,00°. Prosečna vrednost ovog ugla iznosila je -3,88° (tabela

3). Njegova najniža vrednost zabeležena je kod ispitanika podgrupe V, -6,15°, dok je najviša vrednost od – 2,43° izmerena u podgrupi II (tabela 5). Rezultati χ^2-testa pokazali su da je razlika između ovih podgrupa bila statistički značajna ($p < 0,01$).

Upotrebom *Pearson Correlation* testa, u grupi dece i u grupi odraslih, utvrđeni su visokoznačajni pozitivni korelacioni odnosi u vrednostima uglova sagitalnog položaja gornje vilice, SNA, i sagitalnog položaja donje vilice, SNB (tabela 6).

Tabela 6
Rezultati ispitivanja korelacionih odnosa između uglova SNA i SNB proučavanih parametara

Ispitanici	r
Deca	0,85**
Odrasli	0,87**

r – vrednost Pearson-ovog koeficijenta korelacije testa; – visokoznačajna korelacija; SNA – ugao prognatizma gornje vilice; SNB – ugao prognatizma donje vilice**

Diskusija

Naglašena fenotipska heterogenost i varijabilnost neminovno su nametale potrebu za klasifikovanjem „klinički vrlo tipičnih odstupanja sadržanih u kompleksnoj vrsti okluzalne morfologije, grupisanih pod kišobranom termina malokluzija klase III" [2]. U literaturi se pominju mnoge supklasifikacije, originalne ili modifikovane, koje su imale za cilj lakšu, bržu i jednostavniju dijagnostiku i planiranje terapije malokluzije klase III. Međutim, činjenica da ova malokluzija ne predstavlja poseban klinički entitet već postoji kao skup mnogih skeletnih i dentalnih komponenti, ukazuje na to da nijedna supklasifikacija nije sveobuhvatna.

Bishara [1] navodi supklasifikacije koje su dali Stapf (1948), supklasifikujući malokluziju klase III na tipičnu, sa prerazvijenom mandibulom i atipičnu, sa nerazvijenom maksilom, i Tweed (1966), koji deli klasu III malokluzije na dve kategorije: pseudo klasa III, sa normalnim položajem mandibule i nerazvijenom maksilom i skeletnu klasu III sa uvećanom mandibulom. Izveštavajući o etiološkim faktorima malokluzija klase III, Battagel [16] je identifikovao kraniofacijalne osobine koje su u vezi sa fenotipom malokluzije III klase, ne pokušavajući da izvrši supklasifikaciju: oštar ugao kranijalne baze, kratka i retrudirana maksila, prominentna i dugačka mandibula. Međutim, on zapaža da nisu u svim slučajevima ispoljene sve karakteristične osobine. Moyers [17] (1997) klasifikuje malokluziju klase III prema uzroku problema: osealna, dentalna, muskularna. Radi tačnije dijagnoze i plana terapije skeletne klase III, u stomatološkoj, ortodontskoj i hirurškoj literaturi pojavljuju se termini hiperdivergentni i normodivergentni model lica [18]. Neki autori smatraju da supklasifikaciju skeletne klase III sa prisutnim mandibularnim prognatizmom treba izvršiti na osnovu oblika mandibule. Tako, Mackay i sar. [6] identifikuju pet morfoloških podgrupa mandibularnog prognatizma u okviru skeletne klase III. Slično, Hashim i Sarhan [7] vrše supklasifikaciju engleske dece sa mandibularnim prognatizmom u sklopu skeletne klase III i nalaze značajne morfološke razlike na mandibuli. Druga grupa autora sugeriše da je srednji masiv lica odlučujuća komponenta za klasifikaciju pacijenata malokluzije skeletne klase III [8]. I Park i Baik [19] (2001) daju klasifikaciju baziranu na nepravilnostima maksile, po kojoj postoje tri tipa ove malokluzije: tip A je pravi mandibularni prognatizam sa normalno razvijenom maksilom i prerazvijenom mandibulom, tip B se karakteriše prerazvijenom i maksilom i mandibulom sa prednjim ukrštenim zagrižajem, tip C se karakteriše hipoplastičnom maksilom uz postojanje prednjeg ukrštenog zagrižaja.

U našem istraživanju, radi što ranijeg dijagnostikovanja i utvrđivanja dominantnog modela III skeletne klase, primenili smo njenu supklasifikaciju na šest tipova, izvršenu na osnovu sagitalnog položaja vilica prema kranijalnoj bazi [20].

U ispitivanju položaja gornje vilice u odnosu na kranijalne strukture, kao referentne ravni, često se koriste prednja kranijalna baza (SN) i linija prognatizma gornje vilice (NA), definišući ugao SNA. Normalne vrednosti ovog ugla se kreću od 80° do 82°. Kod malokluzije skeletne klase III, čest nalaz je retrognatizam gornje vilice izražen kroz sniženu vrednost ovog ugla, uz ortognatizam ili prognatizam donje vilice. Međutim, meren vrednošću ugla SNA, položaj gornje vilice u skeletnoj klasi III može biti i ortognat, ili čak i prognat. U mnogim studijama, sprovedenim kod dece sa skeletnom klasom III, različitog etničkog porekla, ispitivane su vrednosti ugla SNA, a dobijeni rezultati nisu uvek bili međusobno saglasni. Tako, u svojoj studiji na sirijskoj deci sa skeletnom klasom III, Mouakeh [21] nalazi značajno nižu vrednost ovog ugla od njenih normalnih vrednosti. Slično, Reyes i sar. [22] u kefalometrijskoj studiji na deci bele rase sa skeletnom klasom III, podelivši ih na starosne grupe od 6 do 16 godina, pronalaze da u svim starosnim grupama postoji sniženje vrednosti ovog ugla. Međutim, neki autori ne pronalaze značajnu razliku u veličini ovog ugla pri poređenju njegovih vrednosti kod ispitanika sa skeletnom klasom III i ispitanika sa skeletnom klasom I [23]. Kod naših ispitanika sa skeletnom klasom III, kod odraslih i kod dece, izmerene su prosečne vrednosti ugla SNA ispod njenog normalnog opsega, što ukazuje na retrognatizam gornje vilice u ovim grupama, sa većim stepenom izraženosti kod odraslih ispitanika. Kod ispitanika sa različitim tipovima skeletne klase III, utvrđena je značajna razlika u vrednostima ovog ugla i kod dece i kod odraslih, ($p < 0,01$). U obe grupe, najveći stepen prognatizma gornje vilice zapažen je u podgrupi sa bimaksilarnim prognatizmom (podgrupa IV), dok je retrognatizam gornje vilice bio najizraženiji u podgrupi sa bimaksilarnim retrognatizmom (podgrupa III). Ovaj nalaz je u skladu sa pokazanom visokoznačajnom pozitivnom korelacijom vrednosti uglova prognatizma viličnih kostiju.

Sagitalni položaj donje vilice u odnosu na kranijalne strukture, često se definiše vrednošću ugla inklinacije linije prognatizma donje vilice (NB) prema prednjoj kranijalnoj bazi. To je ugao SNB, čije normalne vrednosti iznose od 78° do 80° i pokazuju na normognatizam donje vilice. U malokluziji skeletne klase III, vrednost ovog ugla je po mnogim autorima povećana i predstavlja razlog dominantnog izgleda donje vilice u facijalnoj morfologiji ove malokluzije [22, 23]. Međutim, rezultati nekih drugih istraživnja pokazuju da vrednosti ovog ugla ne moraju odstupati od normalnih vrednosti, iako kefalometrijska analiza sagitalnih međuviličnih odnosa pokazuje da

se radi o skeletnoj klasi III [19]. To je ustanovljeno i u našem predhodnom istraživanju, gde ispitanici sa skeletnom klasom III nisu imali značajno veće prosečne vrednosti ugla SNB, od onih sa skeletnom klasom I [15]. Rezultati ovog istraživanja pokazali su da je prosečna vrednost ugla mandibularnog prognatizma kod dece sa skeletnom klasom III bila normalna, dok je kod odraslih ispitanika bila nešto iznad njegovih normalnih vrednosti. Ispitanici sa različitim tipovima malokluzije skeletne klase III imali su značajno različite vrednosti ovog ugla i u grupi dece i u grupi odraslih, ($p < 0,01$). U obe grupe najveća prosečna vrednost ugla SNB zabeležena je kod ispitanika sa bimaksilarnim prognatizmom (podgrupa IV), a najmanja u podgrupi sa bimaksilarnim retrognatizmom (podgrupa III). Ovakav nalaz, kao što smo naveli, govori o visokoznačajnoj povezanosti stepena prognatizma viličnih kostiju.

Razlika u stepenu prognatizma gornje i donje vilice, u našem istraživanju, izražavana je vrednošću ugla ANB, koji je najčešće korišćen pokazatelj sagitalnih međuviličnih odnosa. Normalna vrednost ovog ugla je 2°–4°. Njegova snižena vrednost osnovna je karakteristika malokluzije skeletne klase III, te je zato i bio osnovni kriterijum za selekcionisanje naših ispitnih grupa. Studije na deci različitog etničkog porekla pokazuju da je vrednost ovog ugla značajno niža kod dece sa skeletnom klasom III, nego kod dece sa skeletnom klasom I [21–25]. Rezultati naše studije pokazali su da je sagitalni skeletni međuvilični nesklad, izražen nižim vrednostima ugla ANB, u većoj meri prisutan kod odraslih ispitanika sa malokluzijom skeletne klase III, nego kod dece sa istom malokluzijom (tabela 3). Ispitanici sa različitim tipovima malokluzije skeletne klase III, i u grupi odraslih i u grupi dece, imali su značajno različite vrednosti parametara ANB ($p < 0,01$). U obe grupe najveći skeletni sagitalni međuvilični nesklad, očekivano, zabeležen je u podgrupi sa maksilarnim retrognatizmom i mandibularnim prognatizmom, koju je u grupi dece činilo 14% ispitanika, a kod odraslih, više od četvrtine – njih 26%.

Mouakeh [21] kod dece sa skeletnom klasom III, uzrasta od 5 do 12 godina, konstatuje čist retrognatizam gornje vilice kod čak 43,5%, a retrognatizam gornje vilice sa prognatizmom donje vilice kod 29,0%. Isti autor analizira i rezultate drugih autora i izveštava o zastupljenosti različitih morfoloških varijacija ove malokluzije: prema Sanborn-u, kod odraslih, retrognatizam gornje vilice i prognatizam donje vilice postoji kod 9,5% slučajeva, čak kod 33% postoji retrognatizam gornje vilice i ortognatizam donje vilice, a čist prognatizam donje vilice je prisutan kod 45,2%; prema Ellis-u, takođe kod odraslih, retrognatizam gornje vilice i prognatizam donje vilice prisutan je kod jedne trećine ispitivanog uzorka, kod 19,5% postoji čist retrognatizam gornje vilice, a kod 19,2%, čist prognatizam donje vilice; Guyer u uzrastu od 5 do 15 godina, pronalazi kod 25% ispitanika čist retrognatizam gornje vilice, kod 22% retrognatizam gornje vilice udružen sa prognatizmom donje vilice, a kod 20% čist prognatizam donje vilice; Dietrich pronalazi da je čist retrognatizam gornje vilice prisutan u mlečnoj denticiji sa 26%, u mešovitoj sa 44%, a u stalnoj denticiji sa 37%, dok je čist prognatizam donje vilice prisutan 23% u mlečnoj, 20% u mešovitoj i u 31% u stalnoj denticiji [21]. U svojoj velikoj revijalnoj studiji Singh [2] navodi da je kod odraslih pacijenata sa malokluzijom skeletne klase III čest nalaz retrognatizma gornje vilice bez prognatizma donje vilice (20–30%). Međutim, rezultati istraživanja koje su sproveli Reyes i sar. [22] kod dece evroameričkog porekla, uzrasta 6 do 16 godina, sa skeletnom klasom III, u svim starosnim grupama, a naročito grupi od 15 i 16 godina, pokazuju da postoji značajno uvećanje dužine donje vilice i prednje visine lica, bez prisustva retrognatizma gornje vilice.

Rezultati našeg istraživanja pokazuju da je retrognatizam gornje vilice prisutan u gotovo jednakoj meri kod dece i kod odraslih sa skeletnom klasom III, a konstatovan je čak kod 2/3 slučajeva. Kod dece je najčešće udružen sa retrognatizmom donje vilice, dok se kod odraslih najčešće javljao u kombinaciji sa prognatizmom donje vilice i nešto ređe sa njenim retrognatizmom. Prognatizam donje vilice bio je u većoj meri prisutan kod naših odraslih ispitanika, nego kod dece i, generalno, bio je manje zastupljen nego retrognatizam gornje vilice.

Praktični značaj ovih rezultata mogao bi se ogledati u konstataciji da je u našoj populaciji i dece i odraslih u sklopu malokluzije skeletne klase III najčešće prisutan retrognatizam gornje vilice. Iz tih razloga, u lečenju ove malokluzije možemo predložiti što raniju stimulaciju sagitalog razvoja gornje vilice, bilo s ciljem njene definitivne korekcije ili stvaranja povoljnijih skeletnih odnosa koji će doprineti stabilnosti kasnijeg ortognatsko-hirurškog lečenja [12, 26–29].

Zaključak

Najčešća morfološka varijacija sagitalnog položaja gornje vilice kod osoba sa skeletnom klasom III je njen retrognatizam, koji je u jednakoj meri zastupljen kod dece i kod odraslih. Sagitalni položaj donje vilice kod najvećeg broja odraslih bio je prognat, dok je kod dece prognatizam donje vilice prisutan u manjoj meri. Vrednosti uglova prognatizma viličnih kostiju u međusobnoj su visokoznačajnoj korelaciji i kod dece i kod odraslih sa skeletnom klasom III.

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Primljen 31. XII 2010.
Revidiran 1. VI 2011.
Prihvaćen 7. VI 2011.

ORIGINALNI ČLANAK

UDC: 616.314-002-053.2
DOI: 10.2298/VSP101231029T

Zastupljenost karijesa u ranom detinjstvu kod pripadnika različitih etničkih grupa u Južnobačkom okrugu

The presence of early childhood caries among the members of different ethnic groups of the South Bačka District

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Apstrakt

Uvod/Cilj. Karijes u ranom detinjstvu (KRD) predstavlja posebnu formu karijesa mlečnih zuba koja se javlja posle erupcije zuba, brzo napreduje, bez izraženih subjektivnih tegoba i dovodi do mnogobrojnih komplikacija. Cilj rada bio je da se ispita zastupljenost KRD kod dece različite etničke pripadnosti u Južnobačkom okrugu. **Metode.** Istraživanje je sprovedeno kao analitička studija preseka na uzorku predškolske dece, oba pola, i različite etničke pripadnosti. Dijagnoza i klinička forma KRD utvrđena je stomatološkim pregledom prema modifikovanim Wyne-ovim kriterijumima: početni oblik (tip 1) – obuhvata početne karijesne promene bez narušavanja površinske strukture gleđi, umereni oblik (tip 2) – kavitetne karijesne lezije zahvataju ≤ 2 zuba ili površine mlečnih zuba; srednji oblik (tip 3) – kavitetne karijesne lezije zahvataju više od 2 zuba ili dve zubne površine, težak oblik (tip 4) – dva i više gangrenoznih korenova u maksilarnom interkaninom sektoru; težak oblik sa komplikacijama (tip 5) – dva i više gangrenoznih korenova u maksilarnom interkaninom sektoru i prisustvo fistula i/ili apscesa u predelu apeksa korenova zuba. Epidemiološki podaci dobijeni su anketiranjem roditelja pregledane dece. Testiranje značajnosti razlika vršeno je analizom varijanse i χ^2 testom. **Rezultati.** Prevalencija KRD kod dece Južnobačkog okruga, čiji uzrast je bio 41,32 ± 8,57 godina, iznosila je 30,5%. Učestalost KRD iznosila je 50,0% kod romske, 43,8% kod rusinske, 37,8% kod slovačke, 26,1% kod srpske, 25,4% kod mađarske i 27,4% kod dece ostalih nacionalnosti. Učestalost tipa 1, 3, 4 i 5 KRD bila je dva puta veća kod dece koja ne razumeju srpski jezik. **Zaključak.** Učestalost KRD različita je kod dece različitih etničkih grupa. Najviša učestalost KRD ustanovljena je kod dece čiji maternji nije srpski jezik.

Ključne reči:
zub, karijes; prevalenca; deca, predškolska; etničke grupe.

Abstract

Background/Aim. Early childhood caries (ECC) is a special form of caries in primary dentition that affect teeth after eruption, with rapid progression, later symptomatology and numerous complications. The aim of this study was to investigate the frequency of ECC among different ethnic groups of preschool children in the South Bačka District. **Methods.** The survey was performed as a cross-sectional analytical study on the sample of preschool children of both sexes and different ethnic groups in the South Bačka District. The diagnosis and the clinical form of ECC was defined by dental check-ups according to the modified Wyne's criteria: the initial form (type 1) shows carious lesions without disturbing the surface structure of the tooth enamel, the moderate form (type 2) shows carious lesions affecting one or two teeth or their surfaces, the middle form (type 3) shows carious lesions affecting more than two teeth or their surfaces, the severe form (type 4) shows the presence of two and more gangrenous roots in the maxilla intercanini sector, and the severe form with complications (type 5) shows the presence of two and more gangrenous roots in the maxilla intercanini sector with the presence of fistula and/or abscess of tooth root apex. Epidemiological data on the different ethnic groups were obtained by interviewing the parents of the examined children. The tests on significant statistical differences was performed by the variance analysis and χ^2 test. **Results.** The prevalence of ECC in children oped 41.32 ± 8.57 months, of the South Bačka District was 30.5%. The highest disease frequency was found in Roma children (50.0%) as well as in children of Ruthenian nationality (43.8%),

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than in children of Slovakia nationality (37.8%), Serbian (26.1%), Hungerian (25.4%) and other nationalities (27.4%). The frequency of types 1, 3, 4, and 5 ECC was twice as high as in children who do not speak Serbian language. **Conclusion.** The frequency of ECC occurence is different among ethnic groups. The highest frequency of ECC is present among the members of ethnic groups whose native language is not Serbian.

Key words:
dental caries; prevalence; child, preschool; ethnic groups.

Uvod

Mnogobrojna istraživanja [1–4] u svetu ukazuju na to da karijes u ranom detinjstvu (KRD) igra značajnu ulogu u homeostazi, kako oralnog, tako i celokupnog zdravlja deteta. Njegove kliničke manifestacije kao što su: dentalgija, nedostatak zuba, pojava dentoalveolarnih infekcija i oštećenje zametaka stalnih zuba mogu dovesti do oboljenja digestivnog trakta, česte pojave respiratornih infekcija i poremećaja opšteg fizičkog razvoja deteta. Sanacija KRD i njegovih posledica skupim i sofisticiranim materijalima uz učešće visokospecijalizovanog kadra, naročito u opštoj anesteziji, zahteva znatna materijalna sredstva što potencira njegov socioekonomski značaj kako za razvijene, tako i nerazvijene i zemlje u razvoju. Dosadašnja istraživanja KRD ukazala su na kompleksnu interakciju socijalnih, etničkih, kulturoloških, bihevioralnih činilaca i karijesa u ranom detinjstvu [2, 5–9].

Cilj rada bio je da se utvrdi učestalost KRD kod male i predškolske dece različite etničke pripadnosti, koja žive na teritoriji Južnobačkog okruga kao i da se definišu faktori rizika koji utiču na rasprostranjenost ovog oboljenja.

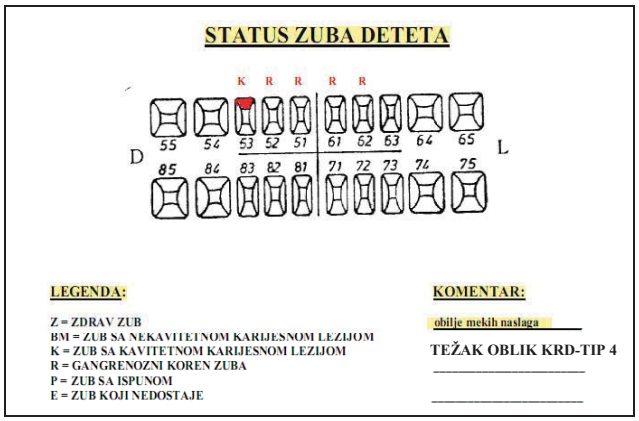
Metode

Istraživanje je sprovedeno kao analitička studija preseka na uzorku dece [10] u i izvan predškolskih ustanova koja borave na sledećim lokalitetima Južnobačkog okruga: Novi Sad, Bačka Palanka, Futog, Kisač, Temerin, Gospođinci, Titel, Selenča, Ruski Krstur, Veliki Rit i Bangladeš. Uzorak je projektovan prostim slučajnim izborom i obuhvatio je predškolsku decu oba pola i različite socijalne i etničke pripadnosti. Optimalan obim uzorka u istraživanju iznosio je 341 dete. Ovaj podatak je izračunat na osnovu pretpostavljene prevalencije KRD u ispitivanoj populaciji dece (30%) odnosno očekivane razlike u učestalosti dece različitih nacionalnosti sa i bez KRD i zadatih vrednosti a = 0,05 i b = 0,20, preciznosti ocene od 5%, uz tačnost od 95% [11].

Stomatološki pregled bio je izvršen pomoću stomatološke sonde i ogledalca uz prirodno osvetljenje. Dijagnostika karijesa vršena je primenom modifikovanih kriterijuma po Wyne-u [12] koji definišu najčešću formu KRD koja se javlja u najranijem uzrastu i obuhvata labiopalatinalne površine mlečnih maksilarnih sekutića i očnjaka.

Na osnovu zahvaćenosti površina zuba karijesom procena težine i klasifikacija KRD vršena je na sledeće tipove[13]: tip 1 = početni oblik („bela mrlja“) – obuhvata početne karijesne promene gleđi bez narušavanja površinske strukture gornjih mlečnih sekutića i očnjaka; tip 2 = umereni oblik – predstavlja kavitetne karijesne lezije koje zahvataju ≤ 2 mlečna zuba ili ≤ 2 površine mlečnih zuba maksilarnog interkaninog sektora; tip 3 = srednji oblik – definiše kavitetne karijesne lezije koje zahvataju više od dva mlečna zuba ili dve zubne površine u maksilarnom interkaninom sektoru; tip 4 = težak oblik – predstavlja prisustvo dva i/ili više gangrenoznih korenova u maksilarnom interkaninom sektoru i tip 5 = težak oblik sa komplikacijama – obuhvata dva i više gangrenoznih korenova u maksilarnom interkaninom sektoru i prisustvo fistula i/ili otoka u predelu apeksa gangrenoznih korenova zuba.

Registracija podataka o prevalenciji karijesa vršena je u originalno dizajniran istraživački karton [13] (slika 1).



Sl. 1 – Istraživački karton

Dijagnostikovane su i numerički evidentirane sledeće nozološke jedinice: Z = zdrav zub, BM = „bela mrlja“ (početni karijes bez prisustva kaviteta), K = zub sa karijesnom lezijom, R = gangrenozni koren zuba, P = zub sa ispunom i E = ekstrahiran ili zub koji nedostaje.

Podaci o etničkoj pripadnosti prikupljeni su putem motivacionog intervjua [14] roditelja pregledane dece i evidentirani u anketne upitnike posebno urađene za ovu studiju [13]. Nepotpuni podaci iz upitnika roditelja i/ili podaci o deci koja zbog bolesti nisu stomatološki pregledana isključeni su iz dalje procene i statističke analize. Istraživanje je odobrila Komisija za etičnost ispitivanja na čoveku Medicinskog fakulteta u Novom Sadu i Etički komitet Stomatološkog fakulteta u Beogradu, uz saglasnost relevantnih državnih institucija i pisanu saglasnost roditelja za učešće njihove dece u ovom istraživanju.

U analizi podataka korišćene su deskriptivne i inferencijalne metode statistike. Od deskriptivnih statističkih parametara analizirana je prevalencija KRD kao i srednje vrednosti ($\bar{x}$) sa njihovim merama varijabiliteta, standardnom devijacijom (SD), standardnom greškom (SE) i koeficijentima varijacije (KV). Testiranje značajnosti razlika vršeno je infe-

rencijalnim metodama: analizom varijanse i χ^2 testom pri čemu je granična vrednost za prihvatanje hipoteze o postojanju međuzavisnosti između testiranih varijabli (nivo statističke značajnosti) postavljena na $p < 0,05$.

Rezultati

Istraživanjem je bilo obuhvaćeno 341 dete Južnobačkog okruga, 191 muškog i 150 ženskog pola, uzrasta 13–72 meseca ($\bar{x} = 41,32 \pm 8,57$ meseci) i različitih nacionalnosti (Srbi, Mađari, Slovaci, Rusini, Romi i dr). Može se zapaziti (tabela 1) da je 104 (30,50%) dece obolelo od KRD, dok 237 (69,50%) nije obolelo. Od ukupno pregledanih 2 046 mlečnih zuba u interkaninom sektoru 1 751 je bilo zdravo, 290 obolelo, a samo 5 zuba je lečeno. Karijes indeks zuba (KIZ) iznosio je 14,42% pri čemu je svako dete imalo prosečno 0,87 obolelih zuba (KIP), odnosno 1,86 zubnih površina zahvaćenih karijesom (KIPS).

Tabela 1
Prevalencija karijesa u ranom detinjstvu (KRD) kod dece predškolskog uzrasta u Južnobačkom okrugu

Parametri	Vrednosti
Ukupan broj dece (n)	341
Pol (n)	
muški	191
ženski	150
Deca sa KRD, n (%)	104 (30.5)
Deca bez KRD, n (%)	237 (69.5)
Zubi maksilarnog interkaninog sektora (n)	
Ukupan broj zuba (n)	2 046
zdravi	1 751
karijesni	290
plombirani	5
kiz (%)	14,42
kip	0,87
kips	1,86

kiz – karijes indeks zuba; kip – karijes indeks prosek; kips – prosečna zubna površina zahvaćena karijesom

U strukturi karijesnih ekstrahovanih plombiranih zuba (KEP) uočena je značajna razlika između karijesnih zuba sa kavitacijom (74,58%), ekstrahovanih (0,00%) i plombiranih (1,69%) zuba, a 23,73% zuba je bilo sa početnim karijesom („bela mrlja“).

Analizirajući uzrast dece (tabela 2) zapaženo je da su najbrojnija deca pripadala starosnoj grupi 37–48 meseci, a zatim 49–60 i 28–36 meseci.

Naša studija, takođe, pokazuje da je u ispitivanom uzorku najviše dece bilo srpske (51,6%) i mađarske nacionalnosti (18,5%), a zatim slovačke (10,9%), rusinske (9,4%) i romske (6,5%) etničke pripadnosti. Neopredeljene u odnosu na etničku pripadnost činilo je 0,9% dece, a 2,3% dece su ubrojena u ostale nacionalnosti.

Tabela 2
Distribucija dece Južnobačkog okruga po starosnim grupama

Uzrast dece (meseci)	Broj dece (%)
13–24	22 (6,45)
25–36	71 (20,82)
37–48	161 (47,22)
49–60	83 (24,34)
61–72	4 (1,17)

U ispitivanom uzorku postojao je trend veće učestalosti karijesa u ranom detinjstvu kod romske (50,0%) i rusinske dece (43,8%) u odnosu na decu slovačke (37,8%), srpske (26,1%) i mađarske (25,4%) nacionalnosti (tabela 3), ali ova razlika nije bilo statistički značajna ($p = 0,07$). Učestalost KRD kod dece koja su pripadala ostalim nacionalnostima i grupi „neopredeljeni“ iznosila je ukupno 27,4%.

Tabela 3
Učestalost karijesa u ranom detinjstvu (KRD) kod dece različitih etničkih grupa u Južnobačkom okrugu

Etnička pripadnost	Deca sa KRD (%)	Deca bez KRD (%)	Ukupno (%)	χ^2
Srpska	26,1	73,9	100,0	
Mađarska	25,4	74,6	100,0	
Slovačka	37,8	62,2	100,0	
Rusinska	43,8	56,2	100,0	
Romska	50,0	50,0	100,0	0,076
Ostali i neopredeljeni	27,4	72,6	100,0	

Korelacija između uzrasta dece i težine KRD prikazana je u tabeli 4. Može se konstatovati da postoji trend veće učestalosti početne karijesne lezije (tip 1) kod dece starosti od 13 do 24 meseca i 25–36 meseci u odnosu na ostale tipove KRD. Kod dece uzrasta 37–48 meseci najučestaliji bio je umeren oblik KRD (tip 2) u odnosu na ostale tipove iste starosne grupe, kao i KRD tip 2 u odnosu na ostale starosne

Tabela 4

Korelacija uzrasta dece i težine karijesa u ranom detinjstvu (KRD)

Težina KRD (%)	Uzrast dece (meseci)				
	13–24	25–36	37–48	49–60	61–72
TIP 1	75,0	40,8	14,5	25,0	0
TIP 2	25,0	25,9	39,6	29,2	0
TIP 3	0	14,8	14,6	16,7	100,0
TIP 4	0	0	12,5	12,5	0
TIP 5	0	18,5	18,8	16,6	0
Ukupno	100,0	100,0	100,0	100,0	100,0

tip 1 – početni oblik („bela mrlja“); tip 2 – umereni oblik; tip 3 – srednji oblik; tip 4 – težak oblik; tip 5 – težak oblik sa komplikacijama

Tabela 5

Jezik sredine kao faktor rizika od pojave i težine karijesa u ranom detinjstvu (KRD) kod dece različitih etničkih grupa Južnobačkog okruga

Težina KRD	Broj dece (%)		p (χ^2 test)
	jezik sredine razume i govori	jezik sredine ne razume	
Bez KRD	72,10	53,20	0,034
TIP 1	6,80	14,90	
TIP 2	10,50	8,50	
TIP 3	3,40	10,60	
TIP 4	2,40	4,30	
TIP 5	4,80	8,50	

tip 1 – početni oblik („bela mrlja“); tip 2 – umereni oblik; tip 3 – srednji oblik; tip 4 – težak oblik; tip 5 – težak oblik sa komplikacijama

grupe ali ove razlike nisu bile statistički značajne (p = 0,391). Srednji oblik KRD (tip 3) bio je najučestaliji u najstarijoj grupi, a težak oblik KRD bez komplikacija (tip 4) kod dece starosti 37–48 meseci i 49–60 meseci. Najveća učestalost teškog oblika KRD sa komplikacijama (tip 5) zapažena je kod dece uzrasta 37–48 meseci. U starosnoj grupi od 49 do 60 meseci najučestaliji bio je umeren tip KRD, a kod dece uzrasta 61–72 meseca srednji tip KRD.

Analizirajući učestalost i težinu KRD uočena je statistički značajna razlika između dece koja razumeju one koja ne razumeju srpski jezik (p = 0,034). Analizom dobijene razlike može se konstatovati da je bez KRD bilo 72,1% dece koja razumeju srpski jezik u odnosu na 53,2% dece koja ga ne razumeju (tabela 5). Takođe, uočena je približno dva puta veća učestalost tipa 1, 3, 4 i 5 KRD kod dece koja ne razumeju srpski jezik u odnosu na decu koja ga razumeju i govore. Izuzetak je KRD tipa 2 koji je bio učestaliji kod dece koja razumeju i govore srpski jezik.

Diskusija

Prevalencija KRD kod predškolske dece u Južnobačkom okrugu može se svrstati u kategoriju srednjih vrednosti rasprostranjenosti ovog oboljenja u odnosu na nisku prevalenciju zapaženu kod dece u Finskoj [15] i Švedskoj [16] i visoku prevalenciju kod dece Hispanoamerikanaca [17] na Srednjem Istoku [18, 19] i u azijskim zemljama[20]. Posmatrajući zemlje u okruženju, Markova [21] i Lulić-Dukić i sar. [22] u svojim radovima navode da su 32% trogodišnjaka u Sofiji i 30% dece uzrasta 2–5 god. u Hrvatskoj oboleli od KRD, što su nešto više vrednosti nego u našoj studiji. Istraživanja Carevića i Vulovića [23] na teritoriji Južnobačkog okruga ukazala su na to da je rasprostranjenost ovog oboljenja kod trogodišnjaka iznosila 22,07%.

Na multietničnost stanovništva Autonomne pokrajine Vojvodine odnosno Južnobačkog okruga ukazuje zvaničan izveštaj Republičkog zavoda za statistiku Srbije (RZSS) [10] sa popisa stanovništva 2002. godine koji definiše 26 nacionalnih entiteta. Naša studija pokazuje da su u ispitivanom uzorku najviše zastupljena deca srpske i mađarske, a zatim slovačke, rusinske, romske i ostalih nacionalnosti. Migratorna kretanja u poslednjoj dekadi dovela su do neznatnih promena u sofisticiranoj klasifikaciji RZSS, ali se to nije značajnije odrazilo na etničku strukturu dece u našem uzorku.

Utvrđeno je da postoje razlike u zastupljenosti KRD kod dece različite etničke pripadnosti. Najviša učestalost KRD uočena je kod dece romske, a zatim rusinske, slovačke i ostalih nacionalnosti, kod kojih je prema našim podacima manje zastupljen srpski jezik. Epidemiološke studije u svetu jasno pokazuju da je povećan rizik od KRD udružen sa etničkom i rasnom pripadnošću [6, 7, 18]. Istraživanja Tinanoff-a i sar. [8] ukazuju na to da pripadnici bele rase imaju veći karijes indeks u poređenju sa crnom rasom, ali manji u odnosu na Hispano amerikance i Mikronežane. Izuzetno visoka prevalencija KRD (55%–72%) zapažena je kod Indijanaca, Hispanoamerikanaca, Afroamerikanaca i starosedelaca Aljaske, što u svojim istraživanjima iznose Huntington i sar. [17] i Douglass i sar. [24]. Da je povećan rizik od nastanka KRD udružen sa etničkom pripadnošću ukazuju Reisine i Psoter [25], Peressini i sar. [26, 27] i Finlayson i sar. [7], a Vachirarojpisan i sar. [28], Hallett i O'Rourke [29] i Psoter i sar. [6] navode da su deca etničkih manjina i imigranata u Holandiji, Engleskoj i Švedskoj imala veću prevalenciju KRD u odnosu na ostalu decu, pri čemu je imigrantski status bio definisan (ne) poznavanjem službenog jezika dotične države. U vezi sa tim neophodno je naglasiti da jezik sredine predstavlja osnov interpersonalne komunikacije, obrazovanja, socijalizacije i ravnopravnog učešća u svim sferama društvenog života. U našoj studiji je zapaženo

da deca koja ne razumeju srpski jezik, nezavisno od etničke pripadnosti, znatno češće obolevaju od KRD nego deca koja ga razumeju i govore. Analizirajući težinu KRD, svi drugi oblici KRD, izuzev umerenog, takođe, zastupljeniji su kod dece koja ne govore srpski jezik. Ovo je najverovatnije rezultat teže dostupnosti zdravstvenih sadržaja i sredstava masovne komunikacije (televizija, radio, štampa) na maternjem jeziku, prvenstveno roditeljima, a samim tim i nižem nivou njihove zdravstvene obaveštenosti i manjom mogućnošću prevencije KRD i drugih oralnih oboljenja kod njihove dece.

Analizirajući uzrast dece i težinu KRD zapažena je veća učestalost početnog karijesa („bela mrlja“) u odnosu na ostale tipove KRD, što potvrđuju i radovi koje su objavili Drury i sar. [30] koji su kod dece uzrasta 6–18 meseci uočili veću prevalenciju bele mrlje – „*white spot*“ lezija u odnosu na kavitetne karijesne lezije. Naša studija, takođe, ukazala je na međuzavisnost uzrasta deteta i težine KRD. Teže forme oboljenja i veći broj obolelih zuba bili su zastupljeniji kod starije dece, bilo zbog porasta ukupnog broja zuba i/ili perzistencije neadekvatnih navika u ishrani, loše oralne higijene i odsustva fluorprofilakse.

Istraživanja u svetu ukazuju na to da se povećan rizik obolevanja od KRD kod dece određenih nacionalnosti može, sa jedne strane, objasniti kulturalnim i verskim normama i shvatanjima vezanim za značaj mlečnih zuba i oralno zdravlje, uopšte [2, 5–9], a sa druge strane niskim socijalnim statusom [16, 17, 25, 31]. Strah od stomatologa, nepoverenje, „sumnja u zdravstveni sistem“ [5] i mišljenje da mlečne zube ne treba lečiti i da je karijes uobičajena pojava među decom predškolskog uzrasta stvaraju *circulus vitiosus* i sve više pogoršavaju postojeće stanje. Takođe, značajno je napomenuti i to da je većina studija koja je istraživala međuzavisnost etničke pripadnosti i prevalencije KRD [2, 3, 5–7, 13, 26, 27] sprovedena kroz programe koji su obuhvatali etničke manjine sa niskim socijalnim statusom („*Head Start children, Medicaid and State Children's Health Insurance Program-SCHIP, Well Child Checkup Program, Women, Infants and Children Program – WIC"* i dr), te je teško razdvojiti kulturalni uticaj etničke pripadnosti od uticaja niskog socijalnog statusa na rasprostranjenost i težinu kliničke slike KRD. Naša studija je obuhvatila decu i njihove roditelje različitog socijalnog statusa, obrazovanja i etničke pripadnosti te su dobijeni rezultati uporedivi sa sličnim istraživanjima KRD u svetu.

Zaključak

Analizom dobijenih rezultata može se konstatovati da postoji razlika u zastupljenosti KRD kod dece različite etničke pripadnosti. Najviša učestalost KRD zapažena je kod romske dece, a zatim kod dece rusinske, slovačke, srpske i mađarske nacionalnosti. Utvrđeno je da postoji direktna korelacija između težine KRD i razumevanja srpskog jezika kod ispitivane dece. Zapažena je statistički značajno viša učestalost „bele mrlje“, srednjeg, teškog i teškog oblika KRD sa komplikacijama kod dece koja ne razumeju srpski jezik, bez obzira na etničku pripadnost.

Neophodno je uz pomoć lokalne samouprave i cele društvene zajednice povećati prohodnost u teško dostupne sredine sa pretežno manjinskim stanovništvom putem promotera zdravstvenog obrazovanja – medijatora iz redova manjinskih zajednica. S obzirom na prostornu neujednačenost i nejednaku dostupnost zdravstvenim informacijama i stomatološkoj službi, u promotivnim aktivnostima i zdravstveno-vaspitnom radu treba angažovati sva raspoloživa zdravstveno-vaspitna sredstva, sredstva masovnog informisanja sa jezički prilagođenim zdravstveno-vaspitnim programima i na taj način smanjiti zastupljenost karijesa u ranom detinjstvu kod pripadnika različitih etničkih grupa.

Zahvalnost

Veliku zahvalnost za stručno-metodološku podršku i sugestije prilikom izrade anketnog upitnika i sociološku analizu dugujem profesorima Stomatološkog fakulteta u Beogradu: Mirjani Ivanović, Ivanki Gajić, Ljubivoju Gvoiću, Mirjani Janjanin, kao i profesoru Mirjani Apostolović sa Stomatološkog odseka Medicinskog fakulteta u Nišu.

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Primljen 31. XII 2010.
Revidiran 15. III 2011.
Prihvaćen 5. IV 2011.
OnLine-first, avgust, 2012.

ORIGINAL ARTICLE

UDC: 616.322/.323-089.97-06::616-005.1
DOI: 10.2298/VSP1212052M

Postadenoidectomy hemorrhage: a two-year prospective study

Krvarenje posle adenoidektomije: dvogodišnja prospektivna studija

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Abstract

Background/Aim. Although postoperative complications are rare, postadenoidectomy hemorrhage is one of the most frequent. The aim of this prospective study was to evaluate the incidence and timing of postadenoidectomy hemorrhage requiring hemostatic control under endotracheal anesthesia. **Methods.** A two-year prospective study of patients undergoing inpatient adenoidectomy, with (n = 462) or without tonsillectomy (n = 589), was undertaken. Surgery was performed in endotracheal anesthesia using an adenoid curette. Every bleeding event which needed procedure in general anesthesia for its treatment was recorded. The timing of postadenoidectomy hemorrhage was classified as primary or secondary. **Results.** Severe bleeding following adenoidectomy with tonsillectomy which needed hemostatic control under endotracheal anesthesia occurred in only 0.19% (2/1051) patients (average age = 7.5 years). Postadenoidectomy hemorrhage was primary in both of the patients. **Conclusion.** Severe postoperative hemorrhage requiring hemostasis under endotracheal anesthesia can be expected in a small number of children undergoing adenoidectomy with tonsillectomy.

Key words:
adenoidectomy; tonsillectomy; postoperative complications; hemorrhage; incidence; child; adult.

Apstrakt

Uvod/Cilj. Iako su postoperativne komplikacije retke, jedna od najčešćih komplikacija je krvarenje posle adenoidektomije. Cilj ove prospektivne studije bio je da se utvrdi učestalost i vreme krvarenja posle adenoidektomije koje zahteva intervenciju u opštoj endotrahealnoj anesteziji. **Metode.** Ovom dvogodišnjom prospektivnom studijom obuhvaćeni su svi bolesnici kojima je načinjenjena adenoidektomija sa (n = 462) ili bez tonzilektomije (n = 589). Adenoidektomija je načinjena u opštoj endotrahealnoj anesteziji pomoću adenotoma. Beleženo je svako postoperativno krvarenje koje je za postizanje hemostaze zahtevalo opštu anesteziju. Vreme krvarenja posle adenoidektomije klasifikovano je kao primarno i sekundarno. **Rezultati.** Krvarenje posle adenoidektomije koje je zahtevalo hemostazu u opštoj anesteziji zabeleženo je kod 0,19% (2/1051) bolesnika prosečne starosti = 7,5 god, kojima je urađena tonziloadenoidektomija. Kod oba deteta krvarenje je bilo primarno. **Zaključak.** Ozbiljno postoperativno krvarenje koje zahteva hemostazu u opštoj endotrahealnoj anesteziji može se retko očekivati kod dece kojoj je učinjena adenoidektomija sa tonzilektomijom.

Ključne reči:
adenoidektomija; tonzilektomija; postoperativne komplikacije; krvarenje; incidenca; deca; odrasle osobe.

Introduction

Adenoidectomy with or without tonsillectomy, remains to be one of the most frequently performed surgical procedures in ear, nose, and throat (ENT) speciality. Although the number of procedures has significantly decreased over the past century, adenoidectomies are still commonly performed surgeries in the pediatric group. Hypertrophy of nasopharyngeal tonsil is the most common cause of nasal obstruction in children. When the obstruction of the nasopharynx causes recurrent infections of upper respiratory tract, chronic otitis media secretoria or sleep apnea, then adenoidectomy, with or without tonsillectomy, is indicated.

Although adenoidectomy is much safer surgical procedure than tonsillectomy, and the postoperative complications are rare, postadenoidectomy hemorrhage is one of the most frequent and potentially life-threatening complications. A recently published study of posttonsillectomy and postadenoidectomy hemorrhage confirms that adenoidectomy has a markedly lower hemorrhage rate than tonsillectomy [1]. Hemorrhage following adenoidectomy can vary from mild bleeding stopping spontaneously to profuse bleeding demanding blood transfusion and surgical procedure in general anesthesia to achieve hemostasis.

The aim of this study was to determine the frequency of postadenoidectomy hemorrhage in the unselected pa-

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tients that required surgical treatment under general anesthesia.

Methods

This two-year prospective study was conducted in the Ear, Nose and Throat (ENT) Department, University Hospital "Zvezdara", Belgrade. The study included 1,051 consecutive patients undergoing inpatient adenoidectomy with (n = 462) or without tonsillectomy (n = 589). There were 1,042 children (≤ 15 years) and 9 adults (> 15 years).

Indications for adenoidectomy were recurrent otitis media, otitis media with effusion and hypertrophy of the adenoids (obstructioon of the upper airways and sleep disorders). There was no patient with coagulation disorder. Prior to surgery, the patients with chronic diseases were in a stable condition.

Children and adults were admitted to the Department one day prior to surgery and had a physical examination, as well as complete blood count and differentiated white blood cell counts, sugar, urea and creatinin levels, bleeding, prothrombin and partial thromboplastin time and urin analysis.

Adenoidectomy was performed in endotracheal anesthesia with oral intubation in Rose's position with retroflexion using a Crowe-Davis gag in suspension. Following palpation of the soft and hard palate, adenoids were removed with an adenotom under optic control *via* a mirror and intraoperative hemostasis was done by temporary application of a cotton swab in nasopharynx.

Traditionally, patients begin to drink liquid two hours after surgery and they can eat soft food. All patients recived antibiotics routinely. In the absence of complications, children and adults were dismissed next day.

Any bleeding events noted by the patients, parents or the staff were recorded regardless the intensity or measures needed for its treatment. Also, timing of bleeding and measure needed for postoperative haemostsis were recorded. The timing of posttonsillectomy hemorrhage was classified as primary (≤ 24 hours) or secondary (> 24 hours).

After discharge, all the patients were advised to return immediately if any bleeding appeared, and were followed up for two months.

Statistical analysis was performed by the IMSL routines for statistical analysis (IMSL Inc, 1989).

Results

This two-year prospective study included 1,051 patients, aged from 1 to 21 years ($\bar{x} \pm SD = 6 \pm 3$ years), undergoing inpatient adenoidectomy with (n = 462) or without adenoidectomy (n = 589). There were 99% children (≤ 15 years) and 1% adults (> 15 years).

In 0.19% (2/1051) of the patients, serious postoperative bleeding requiring the second general anesthesia for revision surgery and postnasal pack occurred. In this two-year study no blood transfusions, ligature of external carotid artery and death were recorded.

Postadenoidectomy hemorrhage was primary in both of the patients and it occurred between 1 and 9 hours ($\bar{x} \pm SD = 4 \pm 2$ h). Secondary hemorrhage was not recorded in this study.

Both of the children who experienced postadenoidectomy bleeding had tonsilloadenoidectomy and were aged 6 and 9 years ($\bar{x} = 7.5$ years). Bleeding was recorded in 0.23% (1/432) of girls and 0.16% (1/619) of boys with no significant difference (χ^2 test, $p = 0.64$).

Discussion

In accordance with the current literature [2–4], present study confirms that bleeding following adenoidectomy is a rare complication of this surgical procedure, and usually occurs in the first postoperative hours. In almost all the patients it is related to adenoid remnants. Usually, residual tissue was found close to the choanas and adjoining the torus tubarius. Removal of these remnants under the second general anesthesia and postnasal pack is the treatment of choice.

Whether performed alone or in association with tonsillectomy, adenoidectomy is one of the most common surgical operations in pediatric otolaryngological practice. The incidence of hemorrhage following adenoidectomy is reported to be from 0% to 0.49% [2–4]. Although postadenoidectomy hemorrhage could be serious and life-threatening, most often it does not require any medical treatment.

Perioperative hemorrhage following curettage adenoidectomy is self-limiting in most cases. In the rare cases of persistent bleeding, it can usually be controlled under direct visualization by temporary packing with a swab impregnated with epinephrine.

Death due to adenoidectomy, with or without tonsillectomy, is caused by bleeding with or without aspiration to the lung, complications of anesthesia and medications, and unidentified causes. Twenty years ago, Rasmussen [5] reported that deaths after adenoidectomy alone have occurred, although the last one was recorded in the Danish medico-legal files in 1939. Recently publish papers described in extremely rare case of postadenoidectomy hemorrhage which required ligature of external carotid artery [6] and one death related to anesthesia and medications [7, 8] and one death due to severe hemorrhage after adenoidectomy [9].

Postadenoidectomy bleeding can almost always be managed by topical compression with postnasal pack, although a second anesthesia can be used if necessary for the removal of any adenoidal remnants. Postnasal packs are usually effective for achieving hemostasis. Standard nasal packs are inserted through the mouth and secured by strips of tape that run anteriorly through both sides of the nose across the columella. Another piece of tape is brought out through the mouth and usually secured to the cheek. In Great Britan, 87% ENT surgeons prefer to manage primary hemorrhage with postnasal packs and 78% would use an overnight post-nasal pack [10].

In the present study, no bleeding was noticed following adenoidectomy without tonsillectomy. Postadenoidectomy bleeding was recorded in the two children who had undergone adenoidectomy with tonsillectomy. This could be due to impatience, and especially to inexperience of younger surgeons who leave residual adenoid tissue close to the choanas or adjoining the torus tubarius.

Conclusion

A low incidence of postoperative bleeding requiring treatment under second endotracheal anesthesia confirms that adenoidectomy continues to be a very secure surgical procedure. Postadenoidectomy hemorrhage usually occurs in the first postoperative hours in children undergoing adenoidectomy with tonsillectomy.

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Received December 31, 2010.
Revised on June 13, 2011.
Accepted on June 14, 2011.

ORIGINAL ARTICLE

UDC: 616.314-77
DOI: 10.2298/VSP110716017P

Influence of balanced occlusion in complete dentures on the decrease in the reduction of an edentulous ridge

Uticaj balansne okluzije kod totalnih proteza na smanjenje redukcije bezubog grebena

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Abstract

Background/Aim. Balanced occlusal arrangement of artificial teeth and balanced occlusion is a specific type of occlusion that preserves the stability of complete dentures. Balanced occlusion comprises realization of tooth contacts at the working side as well as at the balancing side, at the same time. The aim of this study was to assess the influence of balanced occlusal arrangement of artificial teeth on the decrease in reduction of edentulous alveolar ridge. **Methods.** A longitudinal study on 91 fully edentulous patients was conducted using their panoramic radiographs and parameters of vertical dimension of edentulous ridges. All the patients were clinically examined by the same and a qualified dental practitioner. Numerical values of parameters of vertical dimensions of edentulous ridges and lines were statistically processed and compared using the Student's *t*-test. **Results.** Vertical dimensions and heights of edentulous ridges were different after comparison of parameters in complete denture wearers with balanced occlusion and complete denture wearers without bilaterally balanced occlusion, as well as between male and female edentulous patients. Statistically significant differences of heights were established in complete denture wearers' with a set of artificial teeth without balanced occlusion, at the baseline and 12 months after wearing of complete dentures. **Conclusion.** Balanced occlusion is a favored occlusal design in setting of artificial teeth in conventional complete dentures, which preserves edentulous ridge and influence the stability of dentures.

Key words:
denture complete; alveolar bone loss; dental occlusion balanced; radiography, panoramic.

Apstrakt

Uvod/Cilj. Postava veštačkih zuba po balansnoj okluziji (BO) i balansno okluzalno uravnoteženje je posebna vrsta okluzije koja uslovljava i čuva stabilnost totalnih proteza. Balansna okluzija podrazumeva da kontakti veštačkih zuba postoje u isto vreme i na radnoj i na balansnoj strani. Cilj ove studije bio je da se ispita uticaj postave zuba po balansnoj okluziji na sniženje redukcije bezubog grebena. **Metode.** U okviru ove longitudinalne studije ispitan je 91 bezubi pacijent uz korišćenje ortopantomograma i parametara vertikalne dimenzije bezubog grebena. Svi pacijenti bili su i klinički ispitani od strane istog kvalifikovanog stomatologa, specijaliste stomatološke protetike. Numeričke vrednosti parametara vertikalne dimenzije bezubih grebenova bile su statistički obrađene i upoređene korišćenjem Studentovog *t*-testa. **Rezultati.** Vertikalna rastojanja i visine bezubih grebenova bile su različite pri upoređivanju parametara kod pacijenata koji su koristili totalne proteze sa BO i pacijenata koji su imali totalne proteze bez obostrane BO, a takođe i između pacijenata i pacijentkinja. Statistički značajne razlike utvrđene su kod pacijenata sa totalnim protezama u kojima zubi nisu bili postavljeni u skladu sa obostrano uravnoteženom BO, na početku merenja i posle 12 meseci terapije totalnim protezama. **Zaključak.** Balansna okluzija je zadovoljavajući i uspešan okluzalni odnos veštačkih zuba u totalnim zubnim protezama koji utiče na očuvanje bezubog grebena i uslovljava stabilnost totalnih proteza.

Ključne reči:
zubna proteza, totalna; alveolna kost, gubitak; zubna okluzija, balansna; ortopantomogram.

Introduction

The phenomenon of the marked atrophy of the alveolar bone following tooth loss has been termed a "reduction of residual ridges" by Atwood, who considered it a major oral problem entity [1]. These alveolar bone and oral soft tissue changes observed in denture wearers may be an inevitable consequence of the loss of natural teeth, tissue remodelling, occlusal factors, and prolonged denture wear [1–17]. Alveolar bone loss subsequent to a long-term edentulism may be se-

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vere and the process may progress [3, 5, 6, 8–15]. Although generally more pronounced in the mandible and characterized by individual variability in volume and rate, advanced reduction in residual ridges (RRR) presents a significant challenge in prosthetic therapy of edentulous patients [14–17].

Balanced occlusal arrangement is a specific type of occlusion. Occlusion of this kind is used only in the setting of artificial teeth in complete dentures, and usually not related to natural dentition. In the literature, balanced occlusal arrangement is assigned as "balanced occlusion" (BO) comprising realization of contacts at the working side as well as at the balancing side, at the same time [18–21]. Balanced occlusal contacts have convincing advantages, but certain disadvantages, too. The advantages of occlusal balance are preservation of the stability of complete dentures, for the chewing function, and decrease in active loading of supporting tissue and edentulous ridge.

Setting of artificial teeth in balanced occlusal arrangement should cause a reduction in loading to edentulous ridge. In support to this statement are descriptions of significant reductions in edentulous ridges that were evidenced in the literature up to now, particularly for cases which teeth not set in complete dentures according to BO [22–28]. Certain disadvantages of setting of a teeth in balanced occlusal arrangement could be in the fact that changes in chewing pattern are required for denture wearers, which prolongs a period of adaptation to complete dentures [20, 21, 27].

The aim of this study was to assess the influence of balanced occlusal arrangement of artificial teeth on the decrease in the reduction of an edentulous alveolar ridge.

Methods

The patients included in this study were edentulous. The experimental group consisted of 61 conventional acrylic complete denture wearers – 21 men, aged 45–65 years, and 40 women, aged 47–65 years. In complete dentures of the patients of the experimental group, artificial teeth were arranged according to the principles of balanced occlusal arrangement (Figures 1–6). For each patient of the experimental group, complete dentures were constructed in the Clinic of Dental Prosthetic, School of Dentistry, University in Belgrade, following the established procedures using semiad-

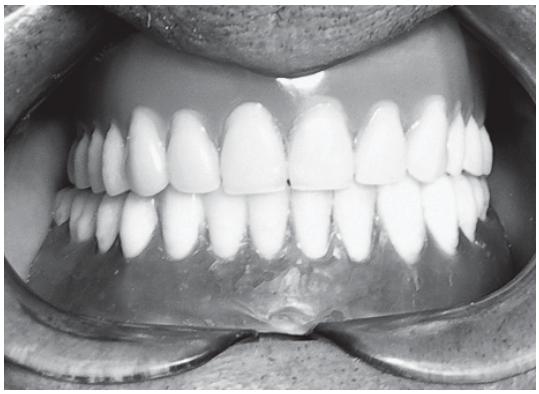


Fig. 1 – Balanced occlusion of artificial teeth in centric position in a patient of the experimental group

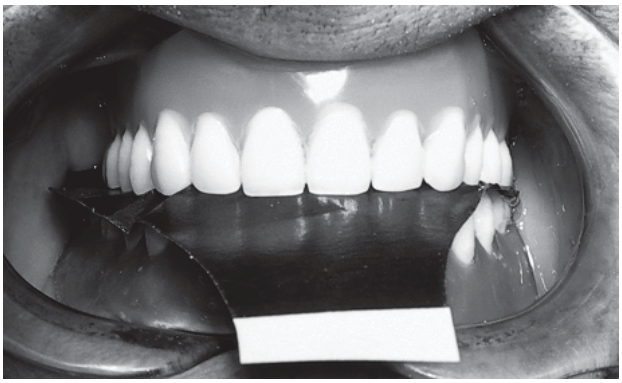


Fig. 2 – Check-in of occlusal contacts of artificial teeth in complete dentures in patient of the experimental group using articulating paper

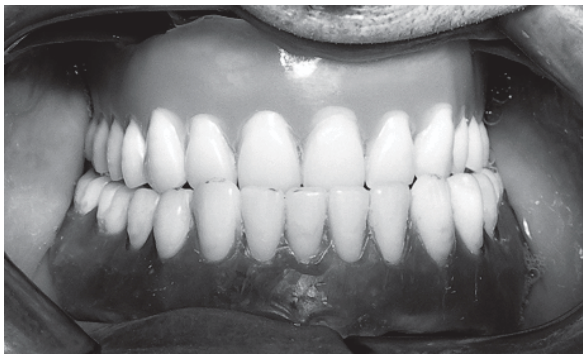
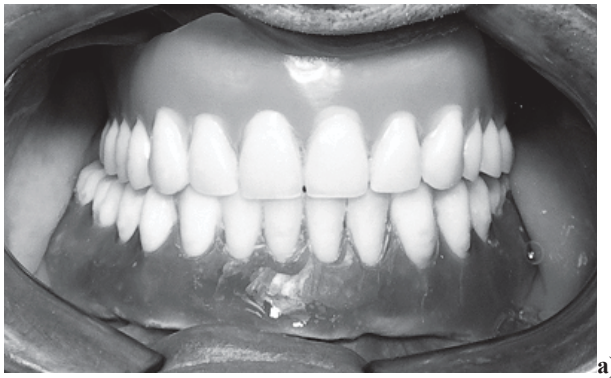
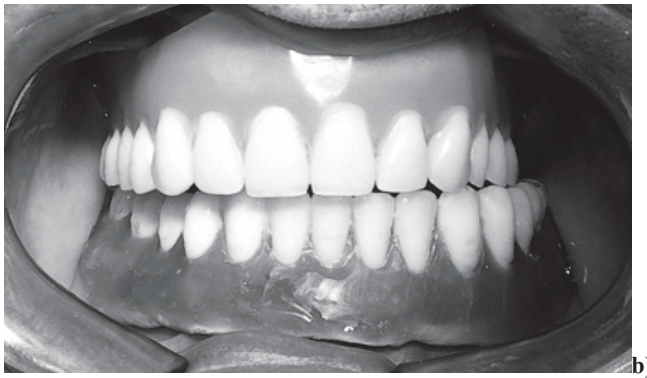


Fig. 4 – Balanced occlusal contacts in propulsion of a patient in the experimental group



a)



b)

Fig. 3 – Balanced occlusion in lateral position

a) to the right side in the mouth of a patient in the experimental group
b) to the left side in the mouth of a patient in the experimental group

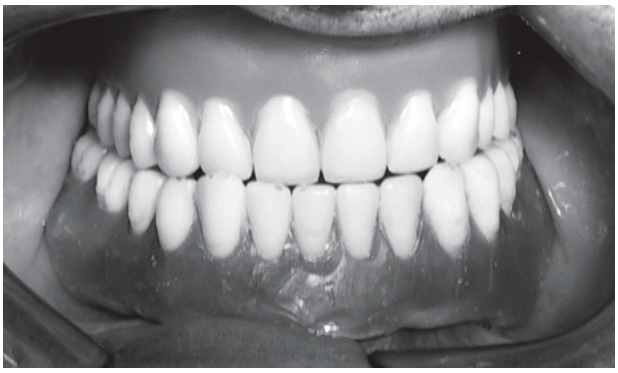


Fig. 5 – Balanced occlusal contacts in lateropulsion of a patient in the experimental group

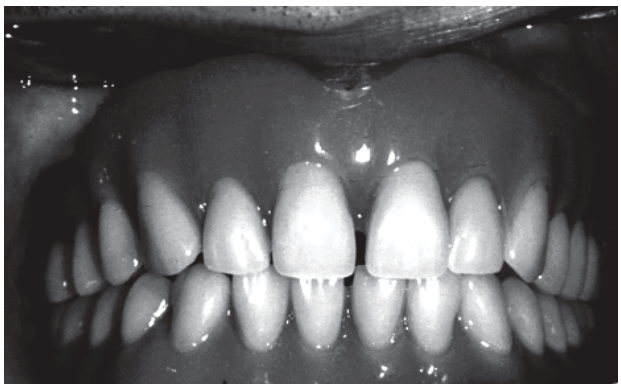


Fig. 6 – Balanced occlusion of artificial teeth in complete dentures of an edentulous woman in the experimental group

justable articulator (Artex, Girbach Dental, Germany). The control group included 30 complete denture wearers – 15 men, aged 50–65 years, and 15 women, aged 49–60 years. In complete dentures of the patients of the control group, artificial teeth were not set appropriately to balanced occlusal arrangement. Complete dentures of the patients of the control group were not fabricated in University Clinic of Dental Prosthetics in Belgrade, and teeth were arranged similar to the occlusal scheme of dentate subjects with unilaterally balanced-grouped occlusal design (Figure 7). The patients of the control group came to the Clinic of Dental Prosthetics, University in Belgrade because of the need for rebasing, relining and correcting their complete dentures, as well as for interest in fabricating a new pair of complete dentures.

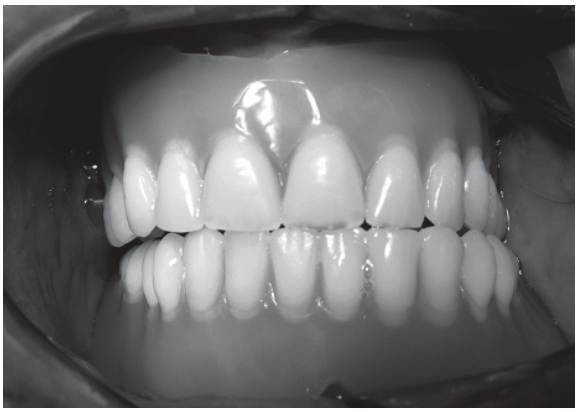


Fig. 7 – Unilaterally balanced occlusion of artificial teeth in complete dentures of a patient in the control group

Standardized panoramic radiographs (Kodak T-MAT G) were made in all of the patients, respecting a determined distance of scale on a plastic chin's holder of orthopantomograph (Orthopantomograph 10-serial no 01492, Siemens, Germany). Two panoramic radiographs were provided for every patient – in a 12-months interval. The baseline of observation was a clinical situation immediately after fabricating and positioning of complete dentures in the mouths of the patients of the experimental group, or after finished rebasing of dentures of patients in the control group. The next measurements were provided 12 months after baseline. A total of 91 patients were assessed in this study, thus 91 panoramic radiographs were analyzed prior to the therapy by complete dentures, and 91 panoramic radiographs were analyzed after 12 months with respect to the baseline. Next, 61 panoramic radiographs were made for the patients of the experimental group after completion of the prosthetic therapy by complete dentures with a balanced occlusal design, in a 12-months period.

Four parameters were analyzed on panoramic radiographs of patients: heights of edentulous ridges of corpuses of mandibles in the areas of mental foramen to the right side and to the left side, and heights of the areas in which were roots of molars to the right and to the left side. Two control parameters were assigned on panoramic radiographs in the regions of distal edges of retromolar pads to the left and to the right side. The parameters were established using vertical lines which were drawn through midpoints of mental phoramens towards lower edges of panoramic radiographs. A segment of each of these lines and a distance from the point on the upper edge of the corpus of mandible to the point on the lower edge of corpus of mandible was adequately marked as V1d to the right side, as well as by V1l. Similarly, lines were drawn from the distal edge of retromolar pads vertically to the lower edge of panoramic radiograph and the segments of these lines were marked as V3d, and V3l, respectively. At the middle of the distance between the lines V1d and V3d, and at the middle of the distance between the lines V1l and V3l vertical lines were drawn in rectangular position with respect to the lower edge of panoramic radiographs, and these lines were assigned as V2d to the right, and V2l to the left. Vertical dimension in this study was measured at 4 sites: in the region of the first missing molars and in the regions of foramen mentale (Figure 8). Heights of lines

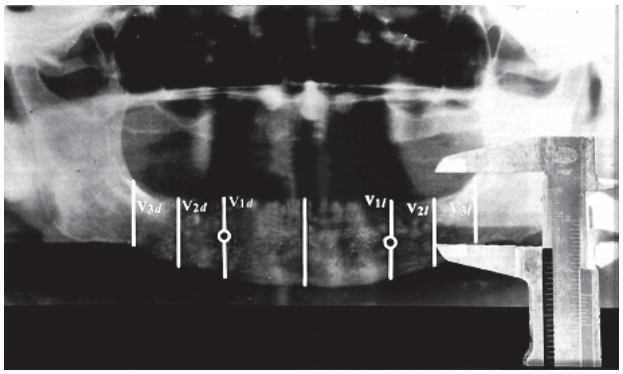


Fig. 8 – Panoramic radiograph of an edentulous patient in the experimental group and parameters of heights of edentulous ridges to the left side – V1l, V2l and V3l and to the right side – V1d, V2d and V3d

which were assigned were measured using a nanometer scale (Manser, Inox, Switzerland) with divisions of 0.1 part of millimeter and with the error of a measurement of 0.05 mm.

A new pair of conventional complete dentures with BO was fabricated for every patient of the experimental group, at the beginning of the measurement.

Numerical values of vertical dimension and lines were statistically processed and compared using the Student's *t*-test.

Results

Numerical values of heights of edentulous ridges were different at the baseline and after a 12-month–observation between the patients of the experimental group and those of the control group.

A vertical dimension and height of edentulous ridges were measured on each panoramic radiograph of the patients. The results and numerical values of heights are shown in tables, separately for edentulous men and women (Table 1). Statistically significant differences of heights were established in the patients of the control group at the baseline and of 12 months after wearing of complete dentures, indicating unsuccessful wearing of their complete dentures and harmful influence of unilaterally balanced occlusal scheme in dentures.

Table 1

The results and numerical values of heights of edentulous ridges for both male and female patients

Patients	Height of segment of mandible's ridge	Experimental group		*p*	Control group		*p*
		$\bar{x} \pm SD$ (mm) at the baseline	$\bar{x} \pm SD$ (mm) other 12 months		$\bar{x} \pm SD$ (mm) at the baseline	$\bar{x} \pm SD$ (mm) other 12 months	
Men	V1d	33.17 ± 3.74	33.04 ± 3.85	ns	30.06 ± 2.57	27.19 ± 3.46	0.06
	V2d	30.28 ± 4.17	30.19 ± 4.32	ns	28.1 ± 2.94	25.42 ± 3.76	0.03
	V1l	33.76 ± 3.89	33.71 ± 3.95	ns	30.21 ± 2.63	27.05 ± 3.58	0.08
	V2l	30.45 ± 4.08	30.41 ± 4.14	ns	28.24 ± 2.89	25.34 ± 3.89	0.02
Women	V1d	30.09 ± 2.96	30.01 ± 3.14	ns	28.27 ± 2.74	24.21 ± 3.07	0.07
	V2d	28.38 ± 2.76	28.42 ± 2.93	ns	26.17 ± 2.98	23.57 ± 2.86	0.02
	V1l	30.02 ± 2.91	29.97 ± 3.05	ns	28.31 ± 2.83	24.16 ± 3.12	0.9
	V2l	28.44 ± 2.81	28.41 ± 3.17	ns	26.14 ± 2.92	23.31 ± 2.93	0.01

ns – no significance

Statistically significant differences in vertical dimensions of edentulous ridges were not established in the experimental group after 12 months of wearing of conventional complete dentures, which indicated success in the therapy by dentures and positive influence of balanced occlusal design of artificial teeth in dentures to preserve the level of alveolar edentulous ridges, and prevent RRR.

Discussion

The problem of reduction in edentulous ridge has been present in the dental literature for many years. A number of authors discussed this problem because of the fact that subsequent consequences are present prior to therapy using complete dentures and rehabilitation of edentulous orofacial system [1–3, 5, 9, 21]. Panoramic radiographs and other dental radiographies have been shown as very reliable and useful in assessment of RRR [4, 6, 7, 10, 17].

Marked atrophy and reduction in the alveolar bone following tooth loss complicate prosthodontic rehabilitation [3, 5, 12, 13, 17, 20, 21]. Difficulties were observed in clinical work in the procedure of impressing edentulous jaws with reduced and resorbed edentulous ridges [19–21]. Many of the problems were obvious in achievement and maintaining the persistence of stability of fabricated acrylic complete dentures. It was particularly shown for lower complete dentures on edentulous mandibles [5, 12, 20, 21]. Edentulous patients with RRR have had serious problems in chewing with complete dentures [13, 16, 20, 21]. Because of all of these reasons it is of crucial importance that the height of edentulous alveolar ridge after tooth extractions remains at the constant level, as much as possible in a long run. Additionally, it is necessary that fabricated acrylic dentures, regarding constant pressures to edentulous ridge, do not cause postponed RRR. Preservation of level of edentulous ridge and alveolar bone after extractions of teeth is of ultimate importance in the therapy and in maintaining of therapeutic effects during wearing complete acrylic dentures.

Based on literature data, it was shown that the most intensive reductions in edentulous ridges, particularly for the lower jaws, happened in mouths of fully edentulous patients in the regions of missing molars [1, 12, 20, 21]. The region of the first missing molar is principally predisposed to reductions. Moreover, it is the fact that this region is very hard to determine in edentulous jaw and difficult to locate precisely on a cast or dental roentgen film, since there are no roots of tooth indicating exact position of the first molar with the region of chewing center [4, 12, 17]. For the reason of determining the region of chewing center and missing first molar, two reliable lines were assigned in this study. These lines in vertical dimension were V1 and V3 lines located mesially and distally respecting region of chewing center of edentulous jaw (Figure 9). The lines V1 were also used in the analysis of reductions in edentulous ridges, concerning the fact that the reduction in an edentulous jaw affects bone structures around mental phoramens, too. The lines V3 were not used in the analysis of reduction in edentulous mandibles, because these lines were drawn from the appearance of retromolar pad which represents the structure of mandible bone that is less predisposed to reductions.

Numerous authors advocated for setting of artificial teeth in complete dentures as the exact copying of position of

teeth prior to extractions. Authors of these clinical studies exposed explanation that the appearance of teeth, arranged the same way as it was in natural dentition prior extraction, offer to a patient an excellent esthetic, as well as much important enhanced subjective feeling of identical position and dimension of dental arch in their mouths without a change in habitual chewing movements of jaws [20, 29–32]. However, it is the fact that characteristic of natural dentition and occlusion is unilaterally balanced occlusion with tooth contacts only at working side which is used in chewing. Most often, in natural dentition there are not contacts at balancing side during chewing on the working side. If this concept of occlusion is applied to setting of artificial teeth in complete dentures, more intensive pressures of artificial teeth at the working side will compromise supporting tissues, and will be a factor for intensive resorption of edentulous ridge influencing RRR [21, 33–37].

The concept of bilaterally BO in complete dentures has to provide that chewing forces and forces in the function of edentulous mouth are applied to the working-ipsilateral and non-working balancing-contralateral side simultaneously, and over all of the surfaces of edentulous ridge, resulting in the decrease in pressure on edentulous jaw, and preventing RRR [27, 38]. This study confirmed the same level of edentulous residual ridge prior the therapy and after the therapy by acrylic complete dentures in the patients of the experimental group. At the same time, the level of residual edentulous ridge significantly decreased in the patients of the control group most probably because of the fact that artificial teeth in their complete denture were not set in accordance with BO (Table 1).

On the basis of this study the concept of balanced occlusal schemes should be applied not only in setting of artificial teeth in conventional complete dentures, but also in construction of complete dentures on implants which are inserted and osseointegrated in edentulous jaws [38, 39].

Conclusion

BO is a favored occlusal design in setting artificial teeth in conventional complete dentures since it preserves edentulous ridge and influences the stability of dentures.

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Received on July 16, 2011.
Revised on September 29, 2011.
Accepted on October 5, 2011.
OnLine-first, May, 2012.

ORIGINAL ARTICLE

UDC: 616.832-001-036.82/.86
DOI: 10.2298/VSP1212061M

Demographic characteristics and functional outcomes in patients with traumatic and nontraumatic spinal cord injuries

Demografske karateristike i funkcionalni oporavak kod bolesnika sa traumatskim i netraumatskim povredama kičmene moždine

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Abstract

Background/Aim. Spinal cord injuries (SCI) could be associated with a significant functional impairment in the areas of mobility, self-care, bowel and bladder emptying and sexuality. The aim of this study was to compare demographic characteristics and functional outcomes of nontraumatic and traumatic spinal cord injury patients. **Methods.** This study was designed as retrospective case series study. A detailed medical history including sex, age, mode of trauma, and clinical and radiological examination was taken for all patients. Hospital records were used to classify the patients according to the following: mechanism of injury, neurological level of injury, functional outcomes, associated injuries, method of treatment, secondary complications and length of stay. The following clinical scores were measured in the patients: American Spinal Injury Association standards (CASTA), Functional Independence Measure (FIM), and Modified Aschworth score (MAS). **Results.** Out of totally 441 patients with spinal cord injury, 279 were traumatic patients (TSCI) and 162 nontraumatic patients (NTSCI); 322 men and 119 women. The mean age of the patients was 46.1 ± 19.9 years. Traumatic and nontraumatic populations showed several significant differences with regard to age, level and severity of lesion. When adjusted for these factors patients with traumatic injuries showed a significantly lower FIM score at admission and significantly better improvement in the FIM score at discharge. The two populations were discharged with similar functional outcome. **Conclusions.** The NTSCI patients in our study were younger, more frequently female, with less complications before rehabilitation and less frequently treated operatively than the TSCI patients. Hospital rehabilitation of the TSCI patients was longer than that of the NTSCI patients, but their functional gain from admission was also higher, so at discharge. Traumatic and nontraumatic spinal cord lesion patients achieved similar results in regard to neurological and functional status.

Key words:
spinal cord, injuries; diagnosis; demography; radiography; treatment outcome; rehabilitation.

Apstrakt

Uvod/Cilj. Povrede kičmene moždine dovode se u vezu sa velikim funkcionalnim poremećajima pokretnosti, samonege, pražnjenja i seksualnosti. Cilj ove studije bio je da se utvrdi korelacija demografskih karakteristika i funkcionalnog oporavka kod bolesnika sa netraumatskim i traumatskim lezijama kičmene moždine. **Metod.** Ova studija urađena je kao retrospektivna studija slučaja. Za podatke o polu, starosti, načinu lečenja, dijagnostičkim pretragama, načinu lečenja, neurološkom nivou lezije, udruženim povredama, sekundarnim komplikacijama, dužini boravka i funkcionalnom oporavku korišćene su istorije bolesti i druga dostupna medicinska dokumentacija. Tokom rehabilitacije bolesnici su bili podrvrgnuti sledećim testovima: *American Spinal Injury Association standards* (ASIA), *Functional Independence Measure* (FIM) i testu *Modified Aschworth score* (MAS). **Rezultati.** Od ukupno 441 bolesnika sa povredom kičmene moždine 279 je bilo sa traumatskim, a 162 sa netraumatskim lezijama. Bilo je 322 muškarca i 119 žena. Prosečna starost bolesnika bila je 46,1 ± 19,9 godina. Traumatske i netraumatske lezije pokazale su nekoliko značajnih razlika u odnosu na pol, nivo i učestalost povrede, udružene povrede, sekundarne komplikacije i funkcionalni

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oporavak. U zavisnosti od navedenih faktora bolesnici sa traumatskim lezijama pokazali su manji FIM skor na prijemu, ali značajno bolji oporavak u FIM skoru na otpustu. Obe grupe bolesnika otpušene su sa približno sličnom funkcionalnom osposobljenošću. **Zaključak.** Kod klinički stabilnih bolesnika tip povreda kičmene moždine ne utiče na prognozu funkcionalnog oporavka. Na prijemu bolesnici sa traumatskim povredama imaju lošiju autonomnost u obavljanju aktivnosti dnevnog života najverovatnije zbog udruženih povreda koje ovi bolesnici imaju. Na otpustu obe grupe bolesnika pokazuju sličan funkcionalni i neurološki oporavak.

Ključne reči:
kičmena moždina, povrede; dijagnoza; demografija; radiografija; lečenje, ishod; rehabilitacija.

Introduction

Spinal cord injuries (SCI) could be associated with a significant functional impairment in the areas of mobility, self-care, bowel and bladder emptying and sexuality [1]. All over the world the incidence of SCI varies between 10.4 and 83 cases per million, per year [2]. In Europe, the incidence is one to 32 per million [2]. The incidence of SCI in the United States is approximately 40 per million, which means around 11,000 new cases each year [3]. Spinal cord injuries can be divided into two subgroups on the basis of their etiology: traumatic (TSCI) and nontraumatic (NTSCI). Within the general population of patients with SCI, traumatic SCI account for the largest portion, and most of the studies on SCI have been conducted with this group of patients. Traumatic SCI occur primarily in young adults, who are in more than half of the cases between 16 and 30 years of age. Men account for about 80% of cases [4]. The percentage of nontraumatic SCI patients is also significant. A previous study on 3,000 patients has reported that one third of SCI patients had a nontraumatic SCI. The fractions of older, female and retired patients are higher in nontraumatic SCI than traumatic SCI group [5]. As nontraumatic SCI patients are usually older, they usually have diabetes, cardiovascular and pulmonary diseases and poor memory. These co-existing health problems could result in a decrease in the efficiency of rehabilitation and in hampering improvement of long-term functionality of the nontraumatic SCI patients [6]. Therefore, traumatic and nontraumatic SCI patients comprise two separate clinical entities, which deserve separate rehabilitation plans in order to improve their functional recovery.

Factors that influence functional outcomes in patients with SCI had been analyzed in considerable number of studies, but are difficult to interpret, since the studies were mostly uncontrolled, observational in character, with short follow-up, with heterogenous cohorts and underpowered. Besides, the complete SCI was variously defined in the last decade, and few studies acknowledged a difference between local neurologic improvement in the area of incomplete lesion and neurologic recovery distal to the injury [7, 8]. Some of the factors were proposed as beneficial for functional recovery after TSCI, like higher ASIA motor score at hospitalization, younger age, level of education, good general health prior to SCI, the absence of spasticity [8] and incompleteness of spinal cord injury [7, 9], but complete evidence is lacking. On the other hand, functional recovery in NTSCI patients was implied to be better with higher Frankel grades of neurologic deficit at first admission to rehabilitation, with younger age and female sex [10, 11], but the associations were weak. Therefore, factors affecting functional outcomes in patients with SCI still remain an unresolved issue.

The hypothesis of our study is that the patients with traumatic SCI, in comparison to the patients with nontraumatic SCI, will have better functional recovery after certain time spent at rehabilitation, regardless the functionality level at admission.

Methods

This study was a retrospective hospital-based analysis of 441 patients with the spinal cord injury admitted to the Clinic for Rehabilitation "Dr M. Zotovic", Belgrade, Serbia, from January 2000 to December 2009. The study sample was consecutive, inclusive of all patients present at the study site satisfying inclusion criteria (diagnosis of a spinal cord injury and signs of neurological lesions of spinal cord) not having exclusion criteria, and non-random. For all the patients, a detailed medical history including sex, age, mode of trauma and clinical and radiological examination was taken. Hospital records were used to classify the patients according to the following: mechanism of injury, neurological level of injury, functional outcomes, associated injuries, methods of treatment, secondary complications and length of stay.

All the enrolled patients satisfied the inclusion criteria. Criteria for exclusion from the study were: any kind of deterioration in the basic condition that resulted in termination of the rehabilitation process, age below 18 years and neurological injury below L3 level at the admission. In total 592 patients were screened, but only 441 enrolled. Of the screened patients, 151 were excluded, due to deterioration of the basic condition (n = 28), age below 18 years (n = 21) and injury below L3 (n = 102).

During rehabilitation the patients were subjected to a series of tests that assessed their functional status and the presence of neurological sequelae after spinal injury: FIM test (Functional Independence Measure), ASIA scale (American Association Impairment scale), MAS (Modified Aschworth Score).

The FIM was a primary functional outcome measure for SCI used in our facility, and its value was determined at admission (AFIM) and discharge (DFIM). The FIM gain is the difference between DFIM and AFIM scores, and it reflects functional improvement. The FIM efficiency is the FIM gain divided by the length of stay (LOS) and reflects the rate of functional improvement [12, 13].

The international standards of the ASIA were used to record motor and sensory levels of the injury. Completeness

of the lesion was recorded according to the AIS. The AIS grades A were defined as a complete motor lesions, and AIS grades B, C and D as incomplete motor lesions [14]. To determine the level of spasticity, we used the MAS.

Recordings were made at the time of admission in the rehabilitation department, as well as at discharge. The data were analyzed for frequency and presented in tables.

For the analysis of primary data descriptive statistical methods were used, as well as hypothesis testing methods. Among the used descriptive statistical methods were the central tendency (arithmetic mean, median), measures of variability (standard deviation) and relative numbers. To test hypothesis about the difference in frequency χ^2-test and Fisher test were used. Mann-Whitney test and *t*-test of exact probability were used for testing hypothesis about difference of arithmetic means. The level of statistical significance in our study was set to 0.05.

Table 1

Characteristics of the patients with spinal cord injuries (SCI)

Parameters	Nontraumatic SCI (n = 162)	Traumatic SCI (n = 279)	*p*
Age, $\bar{x} \pm SD$ (years)	55.5 ± 13.8	40.2 ± 16	< 0.001
Sex, n (%)			
male	92 (56.8)	230 (82.4)	< 0.001
female	70 (43.2)	49 (17.6)	
Polytrauma, n (%)			
no	162 (100)	270 (96.8)	0.03
yes	0(0)	9 (3.2)	
Associated injury, n (%)			
no	162 (100)	172 (61.6)	< 0.001
yes	0 (0)	110 (38.4)	
Complications before rehabilitation, n (%)			< 0.001
no	149 (92)	199 (71.3)	
yes	13 (8)	80 (28.7)	
Methods of treatment, n (%)			
operative	94 (58)	188 (67.4)	0.048
conservative	68 (42)	91 (32.6)	

Results

A total of 441 patients with SCI were included. In the present study, 162 (36.7%) of the SCI patients were in the nontraumatic and 279 (63.3%) were in the traumatic SCI group.

The mean age was found to be 40.2 ± 16 for the traumatic SCI group, and 55.5 ± 13.8 for the nontraumatic SCI group. There was a statistically significant difference between the two groups in terms of age ($p < 0.001$).

Of the total number of patients, 322 (73%) were male and 119 (27%) female. The men were more likely to have traumatic SCI than the women (71.4% *vs* 41.2%) which was statistically significant ($p < 0.001$). In the nontraumatic group of the patients 92 (56.8%) were males, and 70 (43.2%) were females. However, in the traumatic SCI group the proportion of male patients (82.4%, n = 230) was significantly higher than the proportion of female patients (17.6%, n = 49).

When the etiology was analyzed in the traumatic SCI group, it was found that injuries were caused by the following: in 121 (43.4%) patients – falling from a high place, in 114 (40.8%) patients – traffic accident, in 22 (7.9%) patients – gunshot wounding, in 22 (7.9) patients – jump in water headfirst. Etiology of the injury in the non-traumatic SCI group was as follows: tumors in 66 (40.7%) patients, myelopathy in 46 (28.4%) patients, infection in 22 (13.6%) patients, vascular origin in 22 (13.6%) patients, pathological fractures in 3 (1.9%) patients, myelitis in 3 (1.9%) patients and other in 1.8% of the patients. These and other characteristics of the patients with SCI are shown in Table 1.

Of the total number of patients, 255 (57.8%) had an incomplete and 186 (42.2%) a complete spinal cord lesion. Complete lesions were significantly more common in patients with traumatic than nontraumatic SCI (54.1% *vs* 21.6% respectively, $p < 0.001$).

At admission, in all the patients with SCI, the most common were a complete lesion, ASIA A (41.7%), followed by ASIA C (37.4%) and ASIA B (20.9%) type. In the patients with nontraumatic SCI the most common were ASIA C (56.2%), followed by ASIA B (24.1%) and ASIA A (19.8%). In the patients with traumatic SCI, the most frequently preposition occurred ASIA A (54.5%), followed by the ASIA C (26.5%) and ASIA B (19%). There was a statistically significant difference in frequency between complete and incomplete lesions in the groups ($p < 0.001$). Nontraumatic SCI usually had incomplete, and traumatic SCI usually had complete injury (Table 2).

On admission, in all the patients with SCI, the most common were thoracic injuries (42.4%), followed by cervical injuries (37%) and lumbar injuries (20.6%). In the nontraumatic group of the patients the most common were the thoracic injuries (49.4%) followed by cervical injuries (30.9%), and lumbar spine injuries (19.8%). In the traumatic group of the patients the most common were cervical injuries (40.5%), followed by thoracic injuries (38.4%) and lumbar injuries (21.1%). The difference between the groups was statistically insignificant ($p = 0.06$).

Table 2

ASIA score on admission and the type of the lesion

Characteristics of the lesion	Nontraumatic (n = 162)	Traumatic (n = 279)	*p*
Completeness of lesion, n (%)			
incomplete	127 (78.4)	128 (45.9)	< 0.001
complete	35 (21.6)	151 (54.1)	
ASIA on admission, n (%)			
A	32 (19.8)	152 (54.5)	< 0.001
B	39 (24.1)	53 (19)	
C	91 (56.2)	74 (26.5)	
Level of injury, n (%)			
cervical	50 (30.9)	113 (40.5)	0.06
thoracic	80 (49.4)	107 (38.4)	
lumbar	32 (19.8)	59 (21.1)	

ASIA – American Spinal Injury Association

The average duration of rehabilitation for all the patients was 153.7 ± 86.2 days. The minimum duration of rehabilitation was 16, while the maximum was 380 days. The average duration of rehabilitation in the patients with nontraumatic SCI was 126 ± 80.13 days and in the patients with traumatic SCI 169 ± 85.72 days, which was a statistically significant difference ($p < 0.001$).

The mean admission total FIM score was 81.01 ± 12.16 and the mean discharge total FIM score was 104.16 ± 16.37. The mean FIM gain was 23.15 ± 12.68 and the FIM efficiency was 0.21 ± 0.18 points/day.

Comparison of the admission FIM scores between the two groups showed that the traumatic SCI group (77.63 ± 11.31) had significantly lower scores than the nontraumatic SCI group (86.82 ± 11.38) ($p < 0.001$). However, the difference in the discharge FIM scores between traumatic SCI (101.32 ± 18.12) and non-traumatic SCI (109.06 ± 11.27) groups was also statistically significant ($p < 0.001$). The comparison of the FIM gain between the two groups showed that the traumatic SCI group (23.69 ± 13.66) had higher gain than the non-traumatic SCI group (22.24 ± 10.75), but the difference was statistically insignificant ($p = 0,208$).

The mean FIM efficiency was 0.19 ± 0.18 for the traumatic and 0.25 ± 0.18 points/day in the NTSCI patients. There was a statistically significant difference between the FIM efficiency in the nontraumatic and traumatic patients ($p < 0.001$; Table 3).

The mean age in the nontraumatic SCI group was higher than the mean age in the traumatic SCI group, yet we could not say that the patients in the nontraumatic SCI group were very old. The mean age of the nontraumatic SCI group was only 55.5 ± 13.8 years. Etiological factors such as traffic accidents, falling from a high place, jump in water headfirst, which were the most common in the traumatic SCI group, are usually seen in younger people. Therefore, the mean age of traumatic SCI group was lower, only 40.2 ± 16 years.

The proportion of female and male patients was almost the same in the nontraumatic SCI group. However, the number of male patients in the traumatic SCI group was approximately five times higher than the number of females [15, 16]. It is possible that this difference is due to the fact that men take more active part in social and occupational settings, and therefore are in a higher risk for injuries caused by factors like motor vehicle accidents and falling from a high place [16]. In a previously published study, it has been reported that nontraumatic SCI were more common among women and traumatic SCI were more common among men. In our study both nontraumatic SCI and traumatic SCI were more common among men.

Reported lengths of stay (LOS) in the literature show wide ranges. LOS in this study was longer than those reported in studies from the United States (mean 60.8 days), Australia (median 83 days) and Italy (mean 143.1 for traumatic and 91.7 days for nontraumatic injuries). Another

Table 3

Functional outcomes: nontraumatic *versus* traumatic spinal cord injuries (SCI)

Parameters of functional outcomes	Nontraumatic SCI $\bar{x}$ ± SD	Traumatic SCI $\bar{x}$ ± SD	*p*
Duration of stay (days)	126.5 ± 80.1	169.5 ± 85.7	< 0.001
FIM on admission	86.82 ± 11.38	77.63 ± 11.31	< 0.001
FIM at discharge	109.06 ± 11.27	101.32 ± 18.12	< 0.001
FIM gain	22.24 ± 10.75	23.68 ± 13.66	0.208
FIM efficiency (points/day)	0.25 ± 0.18	0.19 ± 0.18	< 0.001

FIM – Functional Independence Measure

Discussion

In this study, demographic characteristics and functional outcomes of the patients were compared and evaluated between the nontraumatic and traumatic SCI patients.

Dutch study reported much longer mean and median LOS (272.9 for traumatic and 240 days for nontraumatic). In addition, different healthcare systems and cultural differences might explain some of the variation in LOS found in the literature [17, 18].

In our study, the leading etiologic factor in the traumatic SCI group was falling from a high place (43.4%), followed by traffic accidents (40.9%), jump in water head first (7.9%) and gunshot wounding (7.9%). In most of the studies on traumatic SCI, the leading cause of injuries were motor vehicle accidents. Kirshblum and O'Connor [19] have reported violent events as the second most common cause of traumatic SCI, as well as the other authors [20, 21]. However, in our study, traffic accident was the second most common cause.

In our study, 57.8% of the patients with SCI had nontraumatic lesions and 42.2% had traumatic lesions. Lower percentages of nontraumatic patients were found in studies from Turkey (32.5%), Italy (25%) and the United States (39%) [21–23].

Etiologic factors in the nontraumatic SCI group in our study were tumor compression (40.7%), myelopathy (28.4%), infection (13.6%) and vascular lesion (13.6%). In a study by McKinley et al. [22, 24, 25] the most common etiologic factors in nontraumatic SCI were found to be spinal stenosis and tumor invasion into the spinal cord. New et al. [26] reported that tumor compression (20.1%) was the first, multiple sclerosis (19.4%) was the second, and degeneration was the third (17.9%) most common cause in nontraumatic SCI patients. In our study, tumor compression was the first and cervical and lumbar myelopathy was the second most common etiologic factor in nontraumatic SCI. The differences in etiological factors of nontraumatic SCI between different countries may be due to social, cultural, and genetic differences [24].

The level of neurological injury in nontraumatic SCI patients was lower than in the traumatic SCI patients. Severity of paraplegia of the patients in the nontraumatic SCI group was significantly higher and they had more incomplete lesions than the patients in the traumatic SCI group. The higher frequency of paraplegia may be due to the differences in the etiologic factors of the two groups. The spinal cord tumor compression, which was the most common cause of nontraumatic SCI, affects mostly the thoracic region and degenerative problems affect mostly the lumbosacral region [26, 27]. Complete lesions and tetraplegia were more frequent in the TSCI group. This finding is in accordance with the results of other studies [1, 19, 20]. A high percentage of complete lesions in our study might be caused by the type of prehospital care, mode of transfer, and whether a trained person does primary medical care and accompanies patients during transportation.

Functionality of nontraumatic SCI patients was better than functionality of the traumatic SCI patients at the time of hospitalization. In general, functional statuses of nontraumatic SCI patients were better than the traumatic SCI patients. Although neurological status was mostly paraplegic and incomplete, functional status was better at the time of the hospitalization in the nontraumatic SCI group; functional gain and functional efficiency were found to be low in this patient group. In other words, the patients with traumatic SCI showed higher improvement of functional status during rehabilitation. This finding is in accordance with the results of other studies [5, 16, 28–31].

The SCI patients should not be grouped only as traumatic and nontraumatic ones, but they should be subcategorized based on etiologic factors, clinic and demographic features and functional results. This categorization should be used for planning of rehabilitation program, definition of targets of the therapy and estimation of the results of the therapy easier, if it were to be based on the etiologic factors (gunshot wounds, traffic accidents, infections, tumors, etc.).

The main limitation of this study lies in the non-random and consecutive sample of patients from only one rehabilitation center. Such design precludes investigation of a variety of rehabilitation methods, since practices in only one center tend to be uniform, and make generalizations difficult since the sample is not representative of wider population of SCI patients.

Conclusion

Although hospital rehabilitation of the traumatic SCI patients was longer than that of the nontraumatic SCI patients, functional recovery of the traumatic SCI patients after rehabilitation was better, regardless the functionality level at admission.

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Received on November 7, 2011.
Revised on January 19, 2012.
Accepted on January 23, 2012.

ORIGINAL ARTICLE

UDC: 616.858-085
DOI: 10.2298/VSP111114028K

Fluoxetine does not impair motor function in patients with Parkinson's disease: correlation between mood and motor functions with plasma concentrations of fluoxetine/norfluoxetine

Fluoksetin ne remeti motornu funkciju kod bolesnika sa Parkinsonovom bolešću: korelacija raspoloženja i motorne funkcije sa koncentracijom fluoksetina/norfluoksetina u plazmi

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Abstract

Background/Aim. Selective serotonin reuptake inhibitors are the most commonly chosen antidepressants in patients with Parkinson's disease (PD). The aim of our study was to assess the influence of fluoxetine (Flu) on motor functions in patients with PD. **Methods.** In this prospective, controlled, open-label study, 18 patients with PD and mild depression [(10 ≤ Hamilton Rating Scale for Depression (HDRS) ≤ 23)] without dementia [(25 ≤ Mini-Mental State Examination (MMSE)] were treated with Flu. Both single and repeated dose effects of Flu were assessed on days 1–80. Plasma concentrations of Flu and norfluoxetine (NORFlu) were correlated with the results of selected motor function performance scores: The Unified Parkinsons Disease Rating Score (UPDRS), Finger Tapping Test (FTT) and Purdue Pegboard Test (PPT). Severity of PD, depression and dementia were evaluated using standard tests [(Hoehn and Yahr stages (HY), activity of daily living (ADL), UPDRS, HDRS, MMSE)]. **Results.** Steady-state for Flu/NORFlu was reached after 18 days of treatment. Such a plateau correlated with significant improvements in both scores of depression and Parkinson's disability (HDRS, UPDRS and ADL, respectively). In addition, FTT and PPT scores also increased until day 18, with further slight fluctuations around the plateau. Optimal motor performances correlated with Flu concentrations of approximately 60–110 μg/L. **Conclusion.** Flu (20 mg/day) significantly reduced depression in PD patients while it did not impair their motor performances. Because substantial placebo effects may arise in studies of PD and depression, large, prospective, randomized, placebo-controlled clinical trials are warranted.

Key words:
parkinson disease; motor activity; depressive disorder; fluoxetine; treatment outcome.

Apstrakt

Uvod/Cilj. Selektivni inhibitori ponovnog preuzimanja serotonina su antidepresivi koji se najčešće koriste u lečenju obolelih od Parkinsonove bolesti (PB). Cilj ovog istraživanja bio je da se proceni uticaj fluoksetina (Flu) na motorne funkcije bolesnika sa PB. **Metode.** U ovom prospektivnom, kontrolisanom, otvorenom kliničkom ispitivanju, 18 bolesnika sa PB i blagom depresijom [10 ≤ Hamiltonova skala za depresiju (10 ≤ HDRS) ≤ 23)], bez demencije [(25 ≤ Mini mental test (MMSE)] lečeni su primenom Flu. Procenjivana su dejstva kako pojedinačne, tako i ponovljene doze Flu od prvog do osamdesetog dana. Plazma koncentracije Flu i norfluoksetina (NORFlu) korelisane su sa rezultatima odeđenih testova za motorne funkcije: skala za procenu težine PB (UPDRS), test spretnosti kucanja (FTT) i Purdue pegboard Test PPT). Izraženost PD, depresije i demencije procenjivane su korišćenjem standardnih testova [(test dnevnih

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aktivnosti (ADL), Hoehn.-Yahr. stadijumi (HJ), HDRS, MMSE)]. **Rezultati.** Ravnotežno stanje za Flu/NORFlu postignuto je 18. dana lečenja. Takav plato u koncentraciji Flu/NORFlu bio je praćen značajnim poboljšanjem rezultata, kako testova za depresiju, tako i za izraženost PB (HDRS, UPDRS i ADL, sledstveno). Dodatno, rezultati FTT-a i PPT-a bili su u porastu do 18. dana, sa blagim fluktuacijama oko platoa. Optimalna motorna postignuća zabeležena su pri koncentraciji Flu od oko 60–110 μg/L. **Zaključak.** Flu (20 mg/dan) značajno redukuje depresiju kod bolesnika sa PB i ne remeti motorne funkcije. S obzirom na mogući placebo efekat u istraživanjima sa PB i depresijom, neophodna su obimnija, prospektivna, randomizovana, placebo-kontrolisana klinička ispitivanja.

Ključne reči:
parkinsonova bolest; motorna aktivnost; depresioni poremećaji; fluoksetin; lečenje, ishod.

Introduction

Depression is the most common and frequently disabling psychiatric condition in patients with Parkinson's disease (PD). Prevalence of depression in patients with PD varies from 7% to 76% depending on the assessment method [1]. Such a depression is mostly persistent or recurrent. It may be accompanied with anxiety, cognitive impairment and may reduce effectiveness of antiparkinson's therapy [2–5]. Depression increases PD patients' disability and significantly reduces their quality of life. Consequently, approximately 50% of patients with PD receive antidepressant therapy [4–7].

Optimal treatment for depression in PD patients has not been established. Several antidepressants were tested in randomized clinical trials without sufficient statistical power (e.g. citalopram, sertraline, fluoxetine, amitriptyline and nortriptyline). Amitriptyline seems to be more effective than fluoxetine in PD patients with severe depression. However, it is not necessarily the first choice for treatment of depression in PD patients, according to the recommendations of the American Academy of Neurology [8]. In addition, the adverse effects of amitriptyline such as orthostatic hypotension, sedation, cognitive and anticholinergic effects might preclude its use and increase the dropout rate in parkinsonians [1, 9, 10].

On the other hand, selective serotonin reuptake inhibitors (SSRIs) are used as a first line treatment of depression 51% of the time [1, 9, 10]**.** In postmortem studies of patients with PD depletion of 5-hydroxytryptamine (5-HT) in the caudate as well as hypothalamus and frontal cortex was reported [11–14], with preferential loss of 5-HT in the caudate compared with the putamen, but with relatively less loss of 5-HT (66%) than dopamine (98%) [15]. Imaging studies *in vivo* have also suggested depletion of 5-HT innervation to the striatum as measured *via* decreased 5-HT transporter binding [16–18]. The loss of striatal 5-HT in PD may be secondary to neurodegeneration within the raphe nuclei as Lewy bodies are seen in the raphe nuclei [19, 20], associated with cell loss [21, 22]. Tauscher et al. [23], 1999, were the first to demonstrate the pharmacodynamic action of the selective 5-HT transporter blocker fluoxetine in the human brain *in vivo*. Meyer et al. [24], 2004, showed that 80% 5-HT transporter occupancy was achievable with SSRI at therapeutic doses in a study on patients with mood and anxiety disorders. Apart from these drug-effects studies, it has been shown that recovery of central serotonergic system after SSRI therapy was associated with reduction of clinical symptoms in 18 depressive subjects using [^{123}I]-CIT and SPECT [25]. All these findings of SSRIs-5-HT transporter occupancy in PET/SPECT studies clearly reflect the pharmacologically induced changes in serotonergic transmission [5, 26].

However, data on the efficacy and safety of SSRIs in PD are still lacking and sufficiently large scale randomised controlled trials are required. Although the introduction of SSRIs offers new opportunities for the treatment of depression in PD, these agents could produce extrapyramidal adverse reactions aggravating parkinsonism [1, 10]. While epidemiological studies have not suggested increased risk of worsening PD using SSRIs for depression [27], almost one hundred detailed reports on extrapyramidal adverse effects linked to SSRIs antidepressants have been published [28, 29].

The influence of Flu on motor performances in PD patients still remains to be clarified. Extrapiramidal side effects of Flu seem to be related to the exacerbation of Parkinson's disability [30]. However, it was also reported that Flu did not increase Parkinson's disability either in retrospective [31] or in prospective studies [32]. Therefore, the authors argue for more systemic and controlled research examining the treatment of depression in patients with PD [1, 33, 34].

The aim of this study was to determine motor performances of PD patients treated with antidepressant Flu and to assess a possible correlation between mood and motor performance scores with plasma concentrations of Flu and its active metabolite, norfluoxetine (NORFlu).

Methods

Efficacy and tolerability of Flu was assessed in the prospective, 80-day, controlled, open-label clinical trial, with blind assessment. Flu was administered to 18 patients with nonfluctuating PD in the early Hoehn and Yahr (HY) stages – as indicator of PD staging only), I and II [35, 36], accompanied with mild depression [(Hamilton Rating Scale for Depression (HDRS): $10 \leq \text{HDRS} \leq 23$)], without dementia [(Mini Mental State Examination (MMSE): ≥ 25)]. These 18 patients were either *de novo* PD patients (PD_0 group, N = 9), or PD patients who were on the stable antiparkinsonian treatment (PD_t group, N = 9), without selegiline, rasagiline and/or dopamine agonists for at least two months prior to Flu.

Patients with secondary parkinsonism, those with the MMSE score < 25 [36], history of stroke, neurological disorder other than PD, or any concomitant serious medical illness, and drug toxicity causing hallucinations, confusional episodes or delirium, were not included in the study. During the study, patients were not allowed to use neuroleptics, seda-

tives, hypnotics or other antidepressants, as well as drugs with potential extrapyramidal adverse effects.

The study was approved by the Ethical Committee of the Faculty of Medicine, University of Belgrade, Serbia. Before entering the study patients gave written informed consent.

All the tests were performed in 18 out of 18 patients on days 11 and 18. Afterwards, 9 out of 18 patients were tested on day 50, and 8 out of 18 patients on day 80 (dropout rates of 50% and 56%, respectively). Therefore results were showed only until day 50.

All the patients were treated with two consecutive dosing regimens.

First, acute treatment with Flu – first day, the patients received Flu, 20 mg per day, at 8 a.m. Evaluation of motor performances and blood sampling for Flu/NORFlu plasma concentration measurement were carried out immediately before the Flu treatment (day 1, 0 h), and 4 h, 6 h and 8 h after the administration of the drug. Flu was than withdrawn for three consecutive days. On the fifth day, patients received 40 mg of Flu at 8 a.m. and all the tests and blood sampling were repeated in the same order (day 5, 0–8 h after administration of the drug). The pattern of blood sampling depends on T_{max} for Flu, ranging from 4 to 8 h after the single dose administration [37] (Figure 1, panel A).

Second, chronic treatment with Flu – in the same patients, regular Flu treatment was initiated (20 mg per day, at 8 a.m.) on day 6 after the beginning of such a therapy, and the motor performances were evaluated on days 11, 18, 50 (steady state for Flu was reached after 18 days of Flu treatment) (Figure 1, panel B).

Two blinded refers evaluated severity of motor impairment using the Unified Parkinson's Disease Rating Scale (UPDRS) – motor score [38], ADL (Schwab and England Activities of Daily Living Score) and computerized version of the quantitative motor test Finger Tapping Test (FTT) [39] and the Purdue Pegboard Test (PPT) [40]. The current severity of depression was evaluated using the 17-item HDRS [41].

Bioanalytical method used for determination of plasma Flu and NORFlu concentrations was high performance liquid chromatography (HPLC) coupled with mass spectrometry (MS). The method used a liquid chromatograph Therm Separation Products Spectra System (Autosampler AS3000, HPLC binary pump P 2000, Degasser SCM 1000), mass spectrometer with electro spray ionization source (Finnigan MAT SSQ 7000 LC/MS – ESI System), Computer Digital UNIX Alpha Station 255. Recovery was very high, not less than 90.8% for Flu and 80.2% for NORFlu. Limit of quantification was 2.5 μg/L for Flu and 10 μg/L for NORFlu, and limit of detection was 1 μg/L for Flu and 5 μg/L for NORFlu. Correlation coefficient was 0.9993 (concentration range of 2.5–250 μg/L), and 0.9989 (concentration range of 10–250 μg/L), for Flu and NORFlu, respectively. Coefficient of variation, calculated for precision, was not higher than 8.33% and 8.83% for Flu and NORFlu, respectively.

The results are expressed as the mean ± standard error of the mean (S.E.M.) of N observations (descriptive statistics). Comparisons between groups were analyzed using the Fisher's exact test, *t*-test, and one-way analysis of variance (ANOVA), when appropriate. In addition, correlation analysis, factor analysis, extraction method (principal component analysis), rotation method (Oblimin with Kaiser normalization) and trend analysis (fitting or least square method) were used.

Results

All the patients were right-handed. Both groups, PD_0 and PD_t, had similar laterality of Parkinson's symptoms (affected right side/affected left side = 6/3).

Among 12/18 patients with the affected right side, there was no significant difference between FFT for the right hand (FTTr) and FTT for the left hand (FTTl) scores, as well as between PPT for the right hand (PPTr) and PPT for the left hand (PPTl) scores (p = 0.66, and 0.89, respectively).

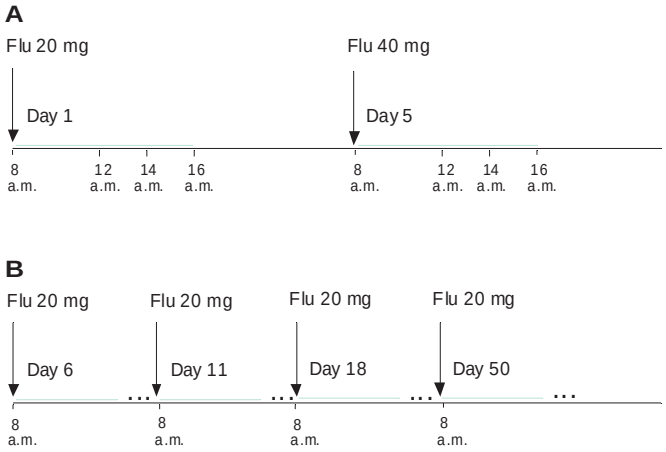

Fig. 1 – Design of the study: acute and chronic treatment of Parkinsonian patients with fluoxetine (Flu) (panel A – acute treatment with Flu, panel B – chronic treatment with Flu)

Among 6/18 patients with affected left side, FTTr was significantly better than FTTl ($p = 0.03$) and PPTr was significantly better than PPTl ($p = 0.02$). In addition, only PPTr score was significantly higher in the left side-affected PD patients comparing to the right side-affected PD patients ($p = 0.03$).

Age, gender and main clinical scores of PD_0- and PD_t-patients are shown in Tables 1 and 2.

Chronic, treatment with Flu: plasma concentrations of Flu and NORFlu increased in a time-related manner (C_{Flu}, and C_{NORFlu}, respectively) (Figure 2).

Table 4 shows plasma concentrations of Flu and NORFlu, as well as motor performance scores for each group assessed during chronic treatment with Flu.

Different patterns of changes were observed in the PD_0 and PD_t patients. In the former case, a sustained increase in both C_{Flu}, and C_{NORFlu} was observed until day 18, i.e. the plateau was reached after 18 days of treatment. In the latter case, plasma concentrations continuously raised until the end of the observation period (day 50) (Table 4). C_{Flu} was significantly higher in PD_0 than in PD_t group after 18 days of treatment (Figure 2A, Table 4).

During chronic treatment with Flu, FTTr scores in the group PD_0 were continuously higher than in the group PD_t, reaching the significance on days 11 and 50 (P = 0.03 and 0.04, respectively) (Table 4). Such a difference was less pronounced regarding FTTl, PPTr and PPTl scores, never reaching statistical significance.

Table 1

Baseline characteristics of patients with Parkinson's disease (PD): group of *de novo* patients without antiparkinson's medication (PDo) and the group with previous stable antiparkinson's therapy (PD_t) (mean ± S.E.M.)

Group of patients	Age (years)	Duration of PD (years)	Previous levodopa therapy		MMSE
			Duration (years)	Dose (mg/day)	
PD_0 (N = 9)	55.7 ± 3.0	2.7 ± 0.9	0	0	28.0 ± 0.6
PD_t (N = 9)	56.0 ± 2.7	3.6 ± 1.1	3.9 ± 0.9	458.3 ± 55.1	27.9 ± 0.9

MMSE – mini mental state examination; PD_0 – *de novo* PD patients; PD_t – PD patients with stable antiparkinsons therapy

Table 2

Staging of Parkinson's disease (PD): the group of patients without antiparkinson's medication (PD0) and the group of patients with stable antiparkinson's therapy (PDt), before (day 1) and on the 18th day of fluoxetine (Flu) medication (day 18 ≈ steady state for Flu) (mean ± S.E.M.)

Group of patients	HDRS		UPDRS		ADL	
	day 1	day 18	day 1	day 18	day 1	day 18
PD_0 (N = 9)	16.4 ± 2.1	10.4 ± 1.9*	26.7 ± 2.9	23.6 ± 3.4	81.7 ± 3.8	85.0 ± 3.4
PD_t (N = 9)	13.6 ± 0.9	8.2 ± 1.1*	29.0 ± 5.1	22.2 ± 4.6*	82.2 ± 3.3	85.6 ± 3.4*

HDRS – Hamilton Depression Motor Scale; UPDRS – Unified Parkinson's Disease Rating Scale; ADL – Schwab and England Activities of Daily Living Score.* – $p < 0.05$, day 0 vs. day 18 (Student's *t*-test for paired data).

Depressive symptoms were similarly reduced after 18 days of Flu treatment in both PD_0 and PD_t patients (Table 2, HDRS scores, $p < 0.05$). At the same time, Parkinson's disability was remarkably improved, especially in PD_t patients (Table 2, UPDRS and ADL, $p < 0.05$, both).

Acute treatment with Flu: there were no remarkable changes in motor function scores (FTT, PPT) after the administration of 20 mg of Flu (day 1), or 40 mg of Flu (day 5) (Table 3).

The groups PD_0 and PD_t differ only in FTTr scores at 0 h and 8 h after the administration of 40 mg of Flu (day 5).

Table 3

Changes in fluoxetine (Flu) and norfluoxwtine (NORFlu) concentrations, and motor function scores (FTT, PPT) during acute treatment with Flu (day 1: 20 mg; day 5: 40 mg) (mean ± S.E.M.)

Day s of Flu treatment		Day 1 of the treatment				Day 5 of the treatment			
Parameter	Group	0 h	4 h	6 h	8 h	0 h	4 h	6 h	8 h
C_{Flu} (μg/L)	PD_0	0	9.58 ± 1.51	11.44 ± 1.31	14.80 ± 0.80	3.24 ± 1.51	19.98 ± 3.30	23.19 ± 1.89	27.40 ± 2.06
	PD_t	0	8.83 ± 1.02	14.76 ± 1.88	16.99 ± 2.28	5.87 ± 1.40	22.71 ± 3.39	25.60 ± 3.90	33.62 ± 2.87
C_{NORFlu} (μg/L)	PD_0	0	0	0	0	0	3.57 ± 1.78	7.48 ± 1.78*	10.48 ± 1.46
	PD_t	0	0	0	0	2.57 ± 1.71	7.72 ± 2.02	11.95 ± 0.48	12.87 ± 0.49
FTTr	PD_0	5.11 ± 0.40	4.91 ± 0.45	5.20 ± 0.35	5.01 ± 0.43	5.44 ± 0.24*	5.31 ± 0.28	5.19 ± 0.31	5.44 ± 0.30*
	PD_t	3.60 ± 0.53	3.55 ± 0.47	3.93 ± 0.43	4.14 ± 0.48	4.17 ± 0.44	4.16 ± 0.45	4.36 ± 0.43	4.16 ± 0.44
FTTl	PD_0	4.25 ± 0.41	4.34 ± 0.36	4.56 ± 0.36	4.57 ± 0.31	4.42 ± 0.24	4.46 ± 0.32	4.49 ± 0.32	4.83 ± 0.41
	PD_t	4.00 ± 0.49	4.05 ± 0.40	4.12 ± 0.46	4.10 ± 0.46	4.21 ± 0.45	4.24 ± 0.42	4.15 ± 0.42	4.38 ± 0.40
PPTr	PD_0	10.33 ± 0.93	11.56 ± 0.96	11.56 ± 1.07	11.22 ± 0.85	11.33 ± 0.94	12.22 ± 0.81	11.78 ± 0.98	11.89 ± 0.92
	PD_t	11.22 ± 1.10	11.56 ± 1.10	11.44 ± 1.09	11.78 ± 1.15	11.44 ± 1.08	11.67 ± 1.24	11.00 ± 1.27	10.89 ± 1.27
PPTl	PD_0	9.22 ± 0.66	9.89 ± 0.66	10.67 ± 0.67	10.44 ± 0.67	10.44 ± 0.75	10.67 ± 0.78	10.78 ± 0.88	10.33 ± 0.87
	PD_t	11.22 ± 0.91	11.89 ± 1.32	10.78 ± 1.28	12.00 ± 1.18	12.00 ± 1.12	12.00 ± 1.30	11.78 ± 1.27	11.44 ± 1.16

PD – Parkinson's disease; PD_0 – *de novo* PD patients; PD_t – PD patients with stable antiparkinson's therapy; C_{Flu}, C_{NORFlu} – plasma concentrations of fluoxetine and norfluoxetine; FTTr, FTTl – Finger Tapping Test for right and left hand; PPTr, PPTl – "Purdue Pegboard Test for right (r) and left (l) hand; * – $p < 0.05$, PD_0 vs. PD_t.

Table 4

Changes in fluoxetine (Flu) and norfluoxetine (NORFlu) concentrations, and motor function scores (FTT, PPT) during chronic treatment with Flu (days 11–80: 20 mg/day) (mean ± S.E.M)

Parameter	Group	Days of Flu treatment		
		Day 11	Day 18	Day 50
C_{Flu} (μg/L)	PD_0	60.73 ± 7.31	112.21 ± 17.95*	87.99 ± 9.88
	PD_t	51.97 ± 6.52	62.34 ± 11.66	94.13 ± 20.54
C_{NorFlu} (μg/L)	PD_0	62.17 ± 12.29	129.17 ± 27.43	106.51 ± 28.73*
	PD_t	60.80 ± 9.45	82.84 ± 11.22	181.74 ± 18.00
FTTr	PD_0	5.51 ± 0.26*	5.56 ± 0.32	5.34 ± 0.45
	PD_t	4.36 ± 0.42	4.47 ± 0.43	3.73 ± 0.80
FTTl	PD_0	4.62 ± 0.35	4.71 ± 0.32	4.78 ± 0.60
	PD_t	4.27 ± 0.40	4.16 ± 0.43	4.11 ± 0.94
PPTr	PD_0	12.22 ± 1.06	12.89 ± 0.88	14.17 ± 0.53
	PD_t	11.61± 0.97	11.83 ± 1.08	14.67 ± 1.76
PPTl	PD_0	10.89 ± 0.83	11.44 ± 0.90	11.81 ± 1.25
	PD_t	12.06 ± 1.12	12.17 ± 1.19	14.50 ± 2.08

PD – Parkinson's disease; PD_0 – *de novo* PD patients; PD_t – PD patients with stable antiparkinson's therapy; C_{Flu}, C_{NORFlu} – plasma concentrations of fluoxetine and norfluoxetine; FTTr, FTTl – finger tapping test for right and left hand; PPTr, PPTl – "Purdue Pegboard Test for right (r) and left (l) hand; * – $p < 0.05$, PD_0 vs. PD_t

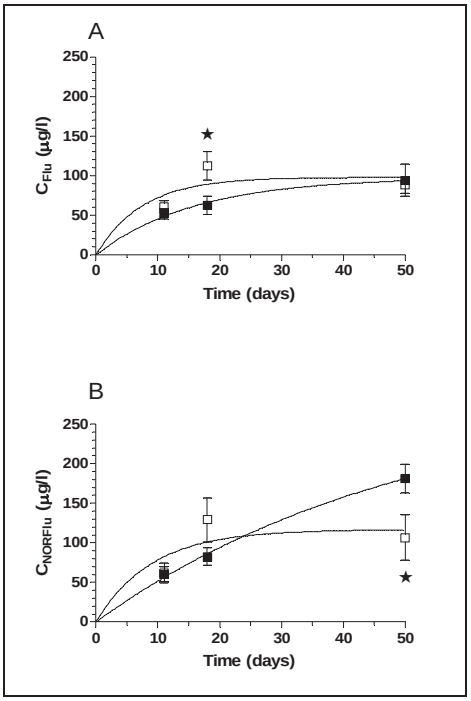

Fig. 2 – Changes in plasma concentrations of fluoxetine (C_{Flu}) and its active metabolite norfluoxetine (C_{NORFlu}) over time (panels A and B, respecively) in PD_0 (□) and PD_t patients (■), during chronic treatment with Flu (days 11–50, 20 mg/day). Each point represents the mean ± S.E.M. of plasma concentrations obtained from 9 separate PD_0 or PD_t patients. *$p < 0.05$, the PD_0 vs. the group PD_t

PD – Parkinson's disease; PD_0 – *de novo* PD patients; PD_t – PD patients with stable antiparkinson's therapy

Of note, the raise in C_{Flu} between days 0 and 18 (the plateau) coincided with the increase in FTT and especially in PPT scores (Tables 3 and 4).

Factor analysis reveals that influence of Flu/NORFlu concentrations increased over time (cummulative data from both PD_0 and PD_t patients; plasma samples were taken on days 0, 5, 11, and 18, six hours after Flu administration). The variance explained by the concentrations of Flu and NORFlu permanently increased from 13.9% (day 5) to 29.9% (day 11) and 37.6% (day 18) of cumulative variance (values of 89.4%, 84.9% and 91.8%, respectively). At the same time, influence of motor function scores decreased over time: variance explained by PPT and FTT scores of 75.5%, 55%, and 54.1% (days 5, 11, and 18, respectively).

PPT and FTT scores significantly correlated on day 11 ($r = 0.62$; $p < 0.01$). In addition, an inverse correlation was found between Flu/NORFlu concentrations and PPT-, but not with FTT scores, on day 18 (r = -0.70 and 0.48, respectively).

Gastrointestinal, cardiovascular side effects and/or insomnia, somnolence and excessive daytime sleepness as adverse reactions to Flu were not reported in the PD patients considered in the study.

Discussion

The major results of our pilot study show that Flu treatment may alleviate depression in PD patients without deterioration of motor function scores. FTT, PPT and UPDRS-motor scores were even improved despite the parallel increase in plasma concentrations of Flu/NORFlu during the first 18 days of the study.

Depression in PD must be properly diagnosed and treated [42]. However, rare reports on the use of various antidepressants in PD patients offer controversial data on their safety regarding motor adverse reactions. Controlled clinical studies confirming the efficacy of Flu in PD patiens and assessing the risk-benefit ratio of such a therapy are still lacking [43].

The broad therapeutic window for Flu is due to its highly variable pharmacokinetics [5, 44–46]. Flu steady state is achieved approximately after 3 weeks (concentrations of approximately 110 μg/L). If plasma concentrations increase above 110 μg/L, the dosage should be adjusted accordingly. Factor analyses indicates that mean Flu concentrations of approximately 60–110 μg/L have the most powerful effect on both PPT and FTT scores, which were significantly improved within that concentration range.

The PPT and FTT are quantitative motor tests. While FTT more reflects motor speed, the PPT is a test for fine motor functions and coordination [40, 47]. Since all the patients were right-handed only among 6/18 patients with affected left side FTTr and PPTr were better than FTTl and PPTl, respectively, pointing to more efficient compensatory mechanisms in dominant hand [48, 49].

The pharmacological profile of fluoxetine is unique among the antidepressants used in PD patients. Fluoxetine is both SSRI agent and a $5HT_{2C}$ antagonist [50]. A recent investigation confirmed that $5HT_{1A}$ agonists and $5HT_{2C}$ antagonists could be important features in treatment of PD. In particular, $5HT_{2c}$ receptors seem to tonically inhibit dopamine release from all three major dopaminergic pathways. Accordingly, $5HT_{2c}$ antagonists could block such an inhibiton, especially in the terminal regions of the nigrostriatal and mesolimbic pathways [51].

Additionally, 5-HT_{2c} receptors are selectively located within substantia nigra pars reticulata (SNr) and medial globus pallidus (GPm) and 5-HT via 5-HT_{2c} receptors is excitatory in the SNr [52–55], which may contribute to the increased activity of these regions in PD. Systemic administration of selective 5-HT_{2c} antagonists to 6-hydroxydopamine-lesioned rodents potentates the antiparkinsonian action of dopamine D_1 and D_2 agonists [56, 57], which is an action mediated via 5-HT_{2c} receptors in the SNr [56]. Thus, 5-HT_{2c} receptor antagonists may improve parkinsonism and drugs with 5-HT_{2c} receptor antagonist action, such as fluoxetine, are unlikely to worsen PD [57].

The pathophysiological mechanisms involved in mood disturbances in PD remain complex. Serotonergic dysfunction has been postulated as such systems are involved in mood disorders in non-PD and the raphe nuclei, as well as hippocampus and prefrontal cortex, appear to be the primary sites affected [58, 59]. Moreover, transcranial ultrasound studies have suggested an association with reduced brainstem raphe echogenicity and nigral hyperechogenicity in patients with depression preceding PD onset compared with nondepressed patients with PD [60]. As the PD disease progresses, Lewy bodies occur with the rostral raphe, thalamus and limbic and cortical regions [15–22, 61], which may result in the mediating of mood disturbances in PD [23–26].

In depression associated with PD, PD-specific pathology, with multiple transmitter deficiencies in mesocortical monoaminergic systems, plays a major role. This includes the mesocorticolimbic dopaminergic projection as well as mesocortical noradrenergic and serotonergic projections. Corticolimbic noradrenergic denervation through cell loss in the locus coeruleus and serotonergic deneravtion *via* serotonergic cell loss in the raphe nucleus are also likely to be important [11–15, 22–26, 62]. Postmortem evidence showed lower density of neurons in the dorsal raphe nuclei in depressed versus nondepressed patients with PD [22] and cerebro-spinal fluid measurement *in vivo* showed reduced serotonin metabolite (5-HIAA) levels in depressed patients with PD [63, 64]. A [11C]-DASB PET study in seven patients with PD with untreated depression showed elevated serotonin transporter binding in the prefrontal cortex compared with non-PD-matched controls [65]. Recently, Politis et al. [66] have reported that the patients with PD with the highest scores for depressive symptoms showed significantly increased [11C]-DSAB binding in the amigdala, hypothalamus, caudal raphe nuclei and posterior cingytlate cortex compared with those patients with low depression scores, though not compared with healthy controls. The [11C]-DSAB binding values in other regions, including the anterior cingulate cortex, caudate, insula, prefrontal cortex, putamen rostral raphe nuclei, thalamus and ventral striatum, were similarly decreased in patients with PD, irrespective of their depressive symptoms scores, compared with the healthy controls. This study demonstrates that depressive symptoms in antidepressant-naïve patients with PD are associated with relatively higher serotonin binding in raphe nuclei and limbic structures. A relative increase in serotonin transporter binding in these regions could reflect either lower extracellular serotonin levels or a disease-related loss of presinaptic serotonergic neurotransmission in contributing to the pathophysiology of PD depression [62, 66].

The phenomenology of depression in PD is also different from that in patints with non-PD with less anhedonia and feeling of guilt [67]. While etiology of depression in Parkinson's disease is unclear (biochemical changes, psychosocial factors and situational stressors have all been implicated), it has an adverse effect on the quality of patients' lives and doctors should ensure that it is diagnosed and properly treated [1, 4, 5, 68].

Therefore, along with improvement on parkinsonian quality of life due to antidepressant activity of SSRI, symptoms such as bradikinesia, hypomima, hypophonia that overlap between depression and parkinsonism could ameliorate because an improvement of mood symptoms [1, 9, 10]. Evenmore, Suzuki et al [69], 2010, suggested that SSRIs such as fluoxetine potentially are therapeutic drugs for non-motor symptoms as well as motor symptoms in patients with PD, since fluoxetine can reverse the downregulation of cell proliferation in the subgranular zone by the unilateral 6-hydroxydopamine lesion.

All these various mechanisms could explain why the improvement in Parkinson's disability scores in our patients coincided with an increase in plasma Flu and NORFlu concentrations during the first 18 days of antidepressive treatment.

Another question is to assess the possible difference between PD_0 and PD_t patients' response to Flu treatment. The beneficial effects of Flu on motor symptoms of PD patients seem to be more pronounced in PD_t group (UPDRS and ADL scores). In addition, PPT scores were mostly higher in PD_t patients during chronic treatment with Flu increasing continuously by the end of the study (day 50). However, the antidepressive efficacy of Flu was similar in both PD groups (HDRS). Also, the statistical significance was rarely observed between those groups regarding motor function scores; FTT values were even somewhat higher in PD_0 patients on days 11 and 50.

According to Taylor et al. [70], depressive symptoms precede those of motor dysfunction in 12–37% of patients with

PD. On the other hand, algorithms for treating depression in PD suggest that optimal antiparkinsonian treatment should precede administration of antidepressants [1, 71]. Our results support such an approach only partially: PD_0 and PD_t groups did not differ in their response to antidepressive therapy, while the influence of Flu on motor functions scores was not consistently related to the pretreatment with antiparkinsonian drugs. Nevertheless, successfull treatment of PD before the administration of antidepressants may diminish overlapping of depressive symptoms and core Parkinson's diasease symptoms [1].

In the present study, we failed to observe any deterioration in motor performance scores of patients with PD that was related to the increase in plasma Flu and NORFlu concentrations. A slight improvement was even observed in all the scores (UPDRS, ADL, FTT and PPT). Similar results were obtained with citalopram, which improved mood but did not decrease motor performance scores in PD treated with levodopa; at the same time, citalopram improved the parkinsonian dysability, bradykinesia and finger taps after one and four months of treatment, both in patients with and without depression [72, 73]. Also, Weintraub et al. [44], 2006, reported that escitalopram was well tolerated, but produced only a partial response in the treatment of major depression in elderly PD patients (mean age of 72.1 years). Two open-label studies suggested that sertraline reduced depression in PD patients, with additional beneficial effect on anxiety, without influencing motor function [74, 75]. Additionaly, Ilic et al. [76] showed that the treatment with sertraline exerts complex modulatory effects on human motor cortex with potential behavioural usefulness. In another open-label study with paroxetine (20 mg/day) given to 33 nondemented depressed PD patients during 6 months, Ceravolo et al. [77], in 2000, reported a significant improvement of depression, as evaluated by HDRS, without influence on parkinsonian symptoms. In only one patient fully reversible worsening of tremor was observed. However, paroxetine frequently may induce tremor as an adverse effect, with a prevalence of 1% to 2%. Chung et al. [78] in 2005, reported that the short-term paroxetine treatment did not alter the motor response to levodopa in patients with PD.

On the other hand, in two retrospective studies worsening of motor symptoms was observed in only small number of PD patients treated with SSRIs [79, 80]. In a prospective study comprising 65 depressed PD out-patients treated with paroxetine (10–20 mg/day) for at least 3 months, two out of 52 patients who completed the study (3%) experienced worsening of parkinsonian symptoms [79]. However, van de Vijver et al. [80], in 2002, observed that the start of SSRI therapy in levodopa users was followed by a faster increase of antiparkinsonian drug treatment. Gony et al. [81], in 2003, failed to find any significant difference in the occurrence of serious extrapyramidal symptoms between different classes of SSRI antidepressant drugs in patients with PD treated with dopaminergic antiparkinsonian drugs. According to the results of several studies [82–84], including our results with Flu, it seems that the benefit of SSRIs outweigh the potential problems due to adverse effects and that they may be considered to be the rational choice in the treatment of depression in PD.

There are several limitations of the study: it was an open-label study without randomization including a small number of patients. As with all nonradomized, open-label trials at tertiary research centers, many non-specific factors, such as relatively long duration of symptoms in *de novo* PD patients, may have influenced the results. However, the quantitative evaluations of motor functions using FTT and PPT significantly improved objectivity and validity of our findings. The observed dropout rates (50% and 56% on days 50 and 80, respectively) are high but fit to the range observed in clinical trials to depression [83].

Conclusion

This pilot study suggests that Flu 20 mg is effective and well tolerated antidepressant in patients with Parkinson's disease. In addition, Flu improved motor function scores in PD patients and such improvement was observed in parallel with the increase in plasma Flu and NORFlu concentrations. Also, the effects of Flu were similar in *de novo* PD patients and in those already treated with antiparkinsonian medications.

Therefore, our results would allow an optimal design for further large, prospective, randomized, placebo-controlled clinical trials that are necessary to evaluate the efficacy and safety of SSRI antidepressants and allow the development of evidence-based guidelines.

Acknowledgment

This work was supported by the Ministry of Science, Republic of Serbia (Project No. 175090).

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Received on November 14, 2011.
Revised on March 1, 2012.
Accepted on May 8, 2012.
OnLine-first, August, 2012.

ORIGINAL ARTICLE

UDC: 57.085::[616.314-089.843:616.716.4-089.851-003.93
DOI: 10.2298/VSP1212076B

The impact of thickness of resorbable membrane of human origin on the ossification of bone defects: a pathohistologic study

Uticaj debljine resorptivne membrane humanog porekla na osifikaciju koštanih defekata – patohistološka studija

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Abstract

Background/Aim. A wide range of resorbable and non-resorbable membranes have been investigated over the last two decades. The barrier membrane protects the defect from ingrowth of soft tissue cells and allows bone progenitor cells to develop bone within a blood clot that is formed beneath the barrier membrane. The membranes are applied to reconstruct small bony defect prior to implantation, to cover dehiscences and fenestrations around dental implants. The aim of this study was to evaluate the influence of human resorbable demineralized membrane (RHDM) thickness on bone regeneration. **Methods.** The experiment, approved by Ethical Committee, was performed on 6 dogs and conducted into three phases. Bone defects were created in all the 6 dogs on the left side of the mandible, 8 weeks after extraction of second, third and fourth premolars. One defect was covered with RHDM 100 μ thick, one with RHDM 200 μ thick, and the third defect left empty (control defect). The histopathological analysis was done 2, 4 and 6 months after the surgery. In the third phase samples of bone tissue were taken and subjected to histopathological analysis. **Results.** In all the 6 dogs the defects treated with RHDM 200 μ thick showed higher level of bone regeneration in comparison with the defect treated with RHDM 100 μ thick and especially with empty defect. **Conclusion**. Our results demonstrated that the thicker membrane showed the least soft tissue ingrowths and promoted better bone formation at 6 months compared with a thinner one.

Key words:
guided tissue regeneration; mandible; dogs; membranes, artificial.

Apstrakt

Uvod/Cilj. U poslednje dve decenije u brojnim eksperimentalnim i kliničkim studijama opisan je i ispitan veliki broj barijernih membrana, njihovih osobina i uloga u vođenoj koštanoj regeneraciji. Membrane imaju za cilj očuvanje krvnog ugruška formiranog u koštanim defektima alveolarne kosti, sprečavanje urastanja epitelnih ćelija u ugrađeni čvrsti zamenik kosti, kao i bolju fiksaciju postavljenih zamenika kosti. Primenjuju se u rekonstrukciji manjih koštanih defekata pre ugradnje implantata, kod dehiscencionih i fenestracionih defekata koji se javljaju kod ugradnje implantata. Cilj rada bio je da se patohistološki ispita uticaj debljine humane resorptivne demineralizovane membrane na osifikaciju koštanih defekata u eksperimentalnoj studiji rađenoj na psima. **Metode.** Eksperimentalna studija, rađena na šest pasa rase nemačkog ovčara po svim etičkim principima, sprovedena je u tri faze. U prvoj fazi izvršena je ekstrakcija drugog, trećeg i četvrtog premolara sa leve strane. Osam nedelja nakon ekstrakcije, formirana su tri defekta na levoj strani mandibule i prekrivena humanom membranom debljine 100 μ, 200 μ, a treći defekt je ostavljen prazan (kontrolni defekt). Patohistološka analiza sprovedena je u periodu nakon dva, četiri, i šest meseci od hirurške intervencije. **Rezultati.** Patohistološkom analizom kod svih šest pasa, ustanovljeno je da su defekti prekriveni humanom membranom debljine 200 μ, imali znatno veći stepen koštane regeneracije u poređenju sa preostala dva defekta. **Zaključak.** Upotrebom humane membrane debljine 200 μ postiže se veći stepen koštane regeneracije.

Ključne reči:
tkivo, vođena regeneracija; mandibula; psi; membrane, veštačke.

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Introduction

Guided bone regeneration, a method that has been generated from guided tissue regeneration, is based on the concept of bone separation from the soft tissue, *i.e.* the prevention of apical migration of gingival epithelial and connective tissue into the defect by the application of a barrier membrane which favors the proliferation of regeneration-capable cells and their differentiation into a desired type of tissue [1]. Guided tissue regeneration is a procedure relevant mostly to natural teeth, while guided bone regeneration is primarily utilized in implantology, where the core of the problem is insufficient amount of bone for implant placement. Five surgical objectives should be realized for the goal of guided bone regeneration to be attained. This involves the use of an appropriate membrane, primary soft tissue healing, closure and maintenance of the membrane-shielded compartment, adaptation and stabilization of the membrane with adjacent bone, and a sufficiently long period of healing [2].

The final aim of the membrane as a barrier is the restitution of supporting tissues (bone or cement, or both, and periodontal ligament), where consequences of inflammation or trauma are present.

The properties of membranes for guided bone regeneration have been described by a number of authors [3–5]. These involve biocompatibility, appropriate barrier ability (mechanical prevention of soft tissue proliferation), tissue integration, immunologic neutrality, preservation of the space for new alveolar bone, and simplicity of application. Such a membrane must hold out against the masticatory forces and tissue tensions of the flap and prevent the collapse of soft tissues and wound space reduction. The property of integration into the tissue guarantees wound stabilization and inhibits epithelial migration [6].

Depending on the reaction to their biologic environment, membranes are divided into resorbable and non-resorbable ones.

Non-resorbable membranes maintain their structure and shape in tissues, and their removal therefore requires additional surgical intervention, which is an additional trauma to the patient, with prolonged wound healing, increased costs, and prolonged overall therapeutic management of the patient. Their appliance is bounded because of the need for secondary surgery, high rate of exposure. These factors can bring about high risk of infection [7].

Resorbable membranes do not require removal after placement, reducing patient discomfort and treatment costs, not to mention the risk of surgical complications. The duration of resorption of these membranes cannot be precisely determined due to their very nature (they can be natural or synthetic), *i.e.* the process of resorption starts as soon as they are placed into the tissue. The literature data about the expected membrane persistence *in vivo* range from 4 weeks to several months [8].

The ability of collagen to stimulate adhesion, hemotaxis, and physiologic degradation of progenitor cells, together with the ability of self-degradation, makes it an ideal material for the construction of membranes. It is also poorly immunogenic, it induces hemostasis, it is capable of augmenting tissue thickness and interacts with a variety of cells during wound healing [9, 10]. Collagen types I and III of bovine or porcine origin are thus the principal components of most of the commercially available membranes.

Cross-linking increases structural stability and slows down the process of degradation. Collagen cross-linking is performed utilizing physical or chemical agents, such as ultraviolet or gamma radiation, hexamethylene, glutaraldehyde diphenyl phosphorylase, and ribose. By way of cross-linking, *in vivo* rate of resorption of collagen materials is controlled and reduced, and mechanical properties are improved. The essence of the process is the formation of various cross-links between certain amino acids and amino acid and carboxylate groups under the action of chemical or physical agents [11].

Membranes with a higher degree of cross-linking stay intact for longer periods of time [12]. Studies have shown that premature membrane resorption or its removal can result in incomplete bone healing, and that is why it has been suggested that membranes applied in guided bone regeneration should have the period of degradation of 3 to 9 months, *i.e.* the period required for bone formation [2].

In the Department of Implantology, a resorbable human demineralized membrane (RHDM) has been developed (patent number 760/02). RHDM is an implantation material of human origin, the structure of which is a barrier to connective tissue ingrowth from the mucoperiostal flap, and the organic composition of which stimulates osteogenesis of the host bone. RHDM is produced by the combination of physical and chemical methods (demineralization of cortical bone with successive removal of lipoproteins) from the calvarial region of human cadavers. Quantitative analyses (Micro-Qeldel, Hidroxyprolin test) and qualitative analyses (collagenase tests, electrophoresis) have shown that the membrane consists of organic components made of collagen type I. The membrane is sterilized using gamma radiation at the end of the production process, what makes it cross-linked.

The role of different thicknesses of RHDM in the regeneration of bone tissue has been insufficiently studied. Studies, especially histopathologic ones in bone regeneration have not been recorded in the literature, and the need for such studies is obvious.

The aim of this paper was to examine histopathologically the impact of thickness of human resorbable demineralized membrane on the ossification of bone defects in an experimental study on dogs.

Methods

This experimental study was performed in the Department of Implantology, Military Medical Academy (MMA), and the Institute for Medical Research, MMA. The experiment had three phases and involved 6 adult German Shepherd dogs, with the medium body (bw) weight of 24.1 kg and average age 5.1 years, abiding by all the ethical principles, as stipulated in the relevant MMA regulations.

The first phase of the experiment

Combelem (intravenous, 0.3 mL/kg bw) and atropin (subcutaneous, 0.03 mL/kg bw) were used as premedication. Ketamine chloride 5% (intramuscular, 0.3 mL/kg bw) was injected 15 minutes after the premedication.

During this short intravenous anesthesia, the extraction of the second, third, and fourth premolar on the left side of the mandible were performed. Extraction wounds were closed using indivudual surgical sutures (Dexon 3,0, Davis & Gack).

Postoperative antibiotic therapy consisted of intravenous administration of 1.600.000 IU of crystalline penicillin for two days. The health of experimental animals was controlled daily, while the dogs were kept in separate boxes and fed soft food. The first phase of the experiment ended eight weeks after extraction.

The second phase of the experiment

Eight weeks after the teeth extraction defects were created. Crystalline penicillin (1.600.000 IU) and metronidazole were administered as prophylaxis. After a supracrestal incizion and vertical relaxations 2–3 mm away from adjacent teeth, a full thickness flap was elevated. On the left side of the lower alveolar ridge occlusally, bone defects were prepared, using a bone trepan (diametar 4 mm), with copious irrigation with sterile physiologic solution. Three bone defects were prepared. The first defect was covered with a resorbable human demineralized membrane 200 μ thick, the second with a 100 μ thick membrane, and the third was left uncovered (control defect).

Each membrane was shaped to cover the defect completely, extending at least 2–3 mm beyond the defect edges. The wounds were closed using individual surgical sutures.

At the end of each of the surgical procedures, and in two more instances afterwards, an analgesic was administered to reduce postoperative pains. The dogs were fed soft diet to the end of the experiment.

The third phase of the experiment

Sacrificing of experimental animals (euthanasia) was done 2, 4, and 6 months after the surgical intervention (two dogs in each instance). Euthanasia was accomplished using the barbiturate thiopental sodium solution (intravenously, 1 g per 30 kg bw). The samples were prepared for histologic analysis. Each sample was cut with a special saw to smaller sections containing the jaw bone defect with appropriate membrane. The cuts were placed in a decalcination solution, and then embedded in paraffin, cut with a microtome in several 5–7 μ thick sections and stained with hematoxyline-eosin (HE), van Gieson, van Kossa, Goldner trichrome, Masson trichrome, PAS, PAS diastase, and toluidine blue.

The preparations were analyzed by light microscopy (Leitz microscope).

The following characteristics were analyzed histopathologically: preservation of compact bone tissue, border between soft tissue elements and newly formed bone trabekulas, the presence of osteocytes in the lacunas, osteoblastic reaction, presence of young blood vessels and fibrocytes, maturity of newly formed connective tissue, and degree of defect-filling with new bone tissue.

Results

The following characteristics were estimated by description of histologic samples according to consecutive criterions given in Table 1.

Table 1

The criterions for estimation of histologic characteristics

Histologic property	Mark		
	"0"	"1"	"2"
Compactness of bone tissue	Not preserved	Partially preserved	Preserved
Borderline between soft tissue elements and newly formed bone	Unclear	Partially clear	Clear
Presence of osteocytes in lacunas	Empty	Partially complete	Complete
Osteblastic reaction	Absent	Moderate	Marked
Presence of young blood vessels, fibroblasts and fibrocytes	Not marked	Partially marked	Marked
Maturity of newly formed connective tissue	Young, immature	Partially mature	Mature
Defect filling with newly formed bone tissue	Defect filling about 1/3	Defect filling less than 2/3	Defect filling more than 2/3

The analyzed characteristics are presented in Tables 2–4.

The Kruscal-Wallis and the Wilcoxon rank-sum tests (Wa test) were used for the purpose of statistical analysis of pathohistologic results.

The methods of guided tissue regeneration, depending on the thickness of applied membranes, were classified as follows: I – resorbable human demineralized membrane 200 μ thick (RHDM200); II – resorbable human demineralized membrane 100 μ thick (RHDM100); and III – defects without a membrane (controls).

According to the calculated statistic values of the Wa test, it was obvious that the RHDM200 method achieved better results in comparison with RHDM100 method in the period of 2 months after the surgery in the 4 analyzed characteristics or in 57.14% cases. In the 3 rest characteristics the effects were the same (Table 5).

Table 2

The comparative histopathologic findings 2 months after the surgical intervention

Property	Method – findings (mark)					
	RHDM 100 μ thick		RHDM 200 μ thick		Control defect	
Compactness of bone tissue	2	Preserved	2	Preserved	0	Not preserved
Borderline between soft tissue elements and newly formed bone	1	Spots of proliferation of soft tissue elements	2	Clear (no ingrowth)	0	Unclear, with soft tissue ingrowth
Presence of osteocytes in lacunas	2	Complete	2	Complete	0	Empty lacunas
Osteblastic reaction	1	Moderate	2	Marked	0	Absent
Presence of young blood vessels, fibroblasts and fibrocytes	2	Marked	2	Marked	2	Marked
Maturity of newly formed connective tissue	0	Immature	1	Partially mature	0	Immature
Defect filling with newly formed bone tissue	0	Less than a third of defect size	1	About a third	0	Less than a third of defect size
Amount:	8		12		2	

RHDM – Resorbable human demineralised membranes

Table 3

The comparative histopathologic findings 4 months after the surgical intervention

Property	Method – findings (mark)					
	RHDM 100 μ thick		RHDM 200 μ thick		Control defect	
Compactness of bone tissue	2	Preserved	2	Preserved	0	Not preserved
Borderline between soft tissue elements and newly formed bone	2	Clear (no ingrowth)	2	Clear (no ingrowth)	0	Unclear, with soft tissue ingrowth
Presence of osteocytes in lacunas	2	Complete	2	Complete	0	Empty lacunas
Osteblastic reaction	2	Marked	2	Marked	1	Moderate
Presence of young blood vessels, fibroblasts and fibrocytes	2	Marked	2	Marked	2	Marked
Maturity of newly formed connective tissue	0	Immature	1	Immature, spotty transformation to mature tissue	0	Immature
Defect filling with newly formed bone tissue	1	About a two third of defect size	2	More than a two third of defect size	0	Less than a two third of defect size
Amount:	11		13		3	

RHDM – Resorbable human demineralised membranes

Tabela 4

The comparative histopathologic findings 6 months after the surgical intervention

Property	Method – findings (mark)					
	RHDM 100 μ thick		RHDM 200 μ thick		Control defect	
Compactness of bone tissue	2	Preserved	2	Preserved	0	Not preserved
Borderline between soft tissue elements and newly formed bone	2	Clear (no ingrowth)	2	Clear (no ingrowth)	1	Unclear, with soft tissue ingrowth
Presence of osteocytes in lacunas	2	Complete	2	Complete	2	Complete
Osteblastic reaction	2	Marked	2	Marked	2	Marked
Presence of young blood vessels, fibroblasts and fibrocytes	2	Marked	2	Marked	2	Marked
Maturity of newly formed connective tissue	1	Immature, spotty transformation to mature tissue	2	Multiplied, mostly mature connective tissue	0	Immature
Defect filling with newly formed bone tissue	1	About a two third of defect size	2	Defect filled for the most part filled with newly formed bone tissue	0	Less than a two third of defect size
Amount:	12		14		7	

RHDM – Resorbable human demineralised membranes

Table 5

The comparative statistics of histological characteristics 2 month after the surgery

Kruskal-Wallis H0: M1 = M2 = M3 H1: M1#M2#M3 *significant level* (α = 0,2)	Compactness of bone tissue Wilcoxon Wa; *significant level* (α = 0,167); *significant interval* (7, 3) I – II H0: M1 = M2, H1: M1 > M2	I – III H0: M1 = M3, H1: M1 > M3	II – III H0: M2 = M3, H1: M2 > M3
H = 3,43/ Hv = 2, α < 3,71	Wa = 5	Wa = 7	Wa = 7
–	–	+	+
	Borderline between soft tissue elements and newly formed bone		
H = 4,57/ Hv = 2,α < 3,71	Wa = 7	Wa = 7	Wa = 7
+	+	+	+
	Presence of osteocytes in lacunas		
H = 3,43/ H*v = 2,α* < 3,71	Wa = 5	Wa = 7	Wa = 7
–	–	+	+
	Osteblastic reaction		
H = 4,57/ Hv = 2,α < 3,71	Wa = 7	Wa = 7	Wa = 7
+	+	+	+
	Presence of young blood vessels, fibroblasts and fibrocytes		
H = 0,00/ Hv = 2,α < 3,71	Wa = 5	Wa = 5	Wa = 5
–	–	-	–
	Maturity of newly formed connective tissue		
H = 3,43/ Hv = 2,α < 3,71	Wa = 7	Wa = 7	Wa = 5
–	+	+	–
	Defect filling with newly formed bone tissue		
H = 3,43/ Hv = 2,α < 3,71	Wa = 7	Wa = 7	Wa = 5
–	+	+	–

(+) presence of a statistically significant difference
(-) absence of a statistically significant difference

In comparison with the control group, the RHDM200 method was significantly better in 6 analyzed characteristics or in 85.71% cases.

In comparison with control group, RHDM100 method achieved better efects in the 4 analyzed characteristics or in 57.14% cases (Table 6).

According to the calculated statistic values of the Wa test, it was obvious that the RHDM200 method achieved better results in comparison with RHDM100 method in the period of 4 months after the surgery in the 2 analyzed characteristics or in 28.57% cases, which presents a decrease with regard to the period of 2 months after the sur-

Table 6

The comparative statistics of histological characteristics 4 month after the surgery

Kruskal-Wallis H0: M1 = M2 = M3 H1: M1#M2#M3 *significant level* (α=0,2)	Compactness of bone tissue Wilcoxon Wa; *significant level* (α=0,167); *significant interval* (7, 3) I–II H0: M1 = M2, H1: M1 > M2	I–III H0: M1 = M3, H1: M1 > M3	II–III H0: M2 = M3, H1: M2 > M3
H = 3,43/ H*v = 2, α*< 3,71	Wa = 5	Wa = 7	Wa = 7
–	–	+	+
	Borderline between soft tissue elements and newly formed bone		
H = 3,43/ H*v = 2, α* < 3,71	Wa = 5	Wa = 7	Wa = 7
–	–	+	+
	Presence of osteocytes in lacunas		
H = 3,43/ H*v = 2, α* < 3,71	Wa = 5	Wa = 7	Wa = 7
–	–	+	+
	Osteblastic reaction		
H = 3,43/ H*v = 2, α* < 3,71	Wa = 5	Wa = 7	Wa = 7
+	–	+	+
	Presence of young blood vessels, fibroblasts and fibrocytes		
H = 0,00/ H*v = 2, α* < 3,71	Wa = 5	Wa = 5	Wa = 5
–	–	-	–
	Maturity of newly formed connective tissue		
H = 3,43/ H*v = 2, α* < 3,71	Wa = 7	Wa = 7	Wa = 5
–	+	+	–
	Defect filling with newly formed bone tissue		
H = 4,57/ H*v = 2, α* < 3,71	Wa = 7	Wa = 7	Wa = 5
+	+	+	+

(+) presence of a statistically significant difference
(-) absence of a statistically significant difference

gery. In the 5 rest characteristics the effects were the same.

In comparison with the control group, the RHDM200 method was significantly better in the 6 analyzed characteristics or in 85.17% cases.

In comparison with the control group, RHDM100 method achieved better efects in the 5 analyzed characteristics or in 71.42% cases, which presents improvement with regard to the period of 2 months after the surgery (Table 7).

The authors also examined and analyzed histopathologically the significance of barrier membranes in bone regeneration in mandibular defects, and found that membrane-covered defects demonstrated significantly better bone healing compared to defects without barrier membranes. The authors stated that barrier membranes increased osteoprogenitor activity of the cells in adjacent bone tissue, enhancing bone regeneration in the mandibular defect. They also found that places regions left uncovered with barrier membrane (control ones) demonstrated incomplete bone regeneration and the presence of soft tissue elements in the newly formed bone.

Table 7

The comparative statistics of histological characteristics 6 month after the surgery

Kruskal-Wallis H0: M1 = M2 = M3 H1: M1#M2#M3 *significant level* (α = 0,2)	Compactness of bone tissue Wilcoxon Wa; *significant level* (α = 0,167); *significant interval* (7, 3)		
	I–II H0: M1 = M2, H1: M1 > M2	I–III H0: M1 = M3, H1: M1 > M3	II–III H0: M2 = M3, H1: M2 > M3
H = 4,57/ H*v* = *2,α* < 3,71 +	Wa = 5 +	Wa = 7 +	Wa = 7 +
	Borderline between soft tissue elements and newly formed bone		
H = 3,43/ H*v* = *2, α* < 3,71 –	Wa = 5 –	Wa = 7 +	Wa = 7 +
	Presence of osteocytes in lacunas		
H = 0,00/ H*v* = *2, α* < 3,71 –	Wa = 5 –	Wa = 5 -	Wa = 5 –
	Osteblastic reaction		
H = 0,00/ H*v* = *2, α* < 3,71 –	Wa = 5 –	Wa = 5 –	Wa = 5 –
	Presence of young blood vessels, fibroblasts and fibrocytes		
H = 0,00/ H*v* = *2, α* < 3,71 –	Wa = 5 –	Wa = 5 –	Wa = 5 –
	Maturity of newly formed connective tissue		
H = 4,57/ H*v* = *2, α* < 3,71 –	Wa = 7 +	Wa = 7 +	Wa = 7 +
	Defect filling with newly formed bone tissue		
H=4,57/ H*v=2,α*<3,71 +	Wa = 7 +	Wa = 7 +	Wa = 5 +

(+) presence of a statistically significant difference
(-) absence of a statistically significant difference

According to the calculated statistic values of the Wa test, it was obvious that the RHDM200 method achieved better results in comparison with RHDM100 method in the period of 6 months after the surgery in the 3 analyzed characteristics or in 42.86% cases, which presents a moderate increase with regard to period the of 4 months after the surgery. In the 4 rest characteristics the effects were the same.

In comparison with the control group, the RHDM200 method was significantly better in the 4 analyzed characteristics or in 57.14% cases.

In comparison with the control group, RHDM100 method achieved better effects in the 4 analyzed characteristics or in 57.14% cases.

Discussion

In the last two decades, numerous experimental and clinical studies have described and examined a large number of barrier membranes, their properties, and roles in guided bone regeneration [13–18].

In the study of Schenk et al. [19] in 1994, guided bone regeneration was investigated on mandibular defects in dogs.

The histopathologic findings in our study are almost identical to the findings of Schenek et al. [19], but their study was less informative as to the course and appearance of bone regeneration in mandibular bone defects.

Schenek et al. [19] described microscopically the three categories of bone tissue encountered in the process of regeneration: 1) woven bone – characterized by netlike distribution of bone trabeculas, with plenty of spheric osteocytes and rich blood supply to the connective tissue between bone trabeculas; 2) fibrillar bone – consists of parallel collagen fibers, with less osteocytes. Growing bone forms a primary osteon, positioned in periostal and endosteal apposition; 3) lamellar bone – a mature bone with lamellar structure.

Based on these findings, the authors concluded that bone regeneration was incomplete after 4 months, and suggested longer periods of healing [19].

In our study, histopathologic analysis of the defects with human resorbable demineralized 100 μ and 200 μ thick membranes two months after surgery, demonstrated a clear-cut borderline between soft tissue elements and compact bone exclu-

sively in the defects covered with 200 μ thick human membrane. Compared to the others, this was also the defect which was best filled with immature connective tissue, rich in fibroblasts, and with numerous newly formed, thin walled, and dilated blood vessels. In the young connective tissue, newly formed shorter and thinner fibrillar bone trabeculas could be observed, in a net-like distribution pattern and with osteoblastic reaction.

Four months after surgery, histopathologic analysis revealed that the borderline between soft tissue and compact bone in both membrane-covered defects was clear, and that there was no proliferation of connective tissue elements. Immature connective tissue was growing mature, and a considerable amount of trabecular bone with pronounced osteoblastic reaction could be seen. After four months, the defect covered with 200 μ thick human membrane was the fullest one (although just slightly more than half of its size).

Six months after the surgery, histopathologic analysis demonstrated that the borderline between bone and soft tissue was clear and without proliferation and ingrowth of soft tissue elements into the bone tissue. In the central area, beneath the compact bone, enlarged, mostly mature tissue could be seen, rich in fibroblasts and fibrocytes, and with numerous young, thin-walled, dilated blood vessels. In the connective tissue, there were thicker and thinner bone trabeculas, with osteocytes in the lacunas, and with marked osteoblastic reaction. Both membrane covered defects were for the most part filled with newly formed bone tissue. The defect covered with 200 μ membrane was almost completely filled.

As for the control defects, a borderline between bone and connective tissue was poorly defined along the whole of its length. Connective tissue elements penetrated deeply into the bone tissue, so that the remnants of bone trabeculas were seen in the connective tissue, having an almost „embedded“ appearance. The region of preserved bone tissue beneath the soft tissue was very narrow as the consequence of proliferation of soft tissue into the bone and its destruction. In animals sacrificed 2 months after surgery, we could see mostly the remnants of bone trabeculas, with empty lacunas and without osteocytes. Between the fragments of bone trabeculas, the growth of immature connective tissue could be observed, with rich blood supply and with numerous fibroblasts and fibrocytes. Four months after the bone defect had been made, clear connective tissue proliferation could be seen, rich in fibroblasts and young blood vessels, as well as a considerable number of new bone trabeculas with the signs of osteoblastic reaction. The defect was for the most part filled with immature, amply vascularized connective tissue. In the sections taken 6 months after sacrificing, in the newly formed, well vascularized connective tissue, a considerable number of thin, shorter or longer fibrillar bone trabeculas were seen, with marked osteoblastic reaction and in a netlike distribution pattern. Soft tissue ingrowth into the bone was obvious. Bone defects were filled to a higher degree compared to the defects seen after 4 months, although not completely as yet; and after 6 months, the defects were filled for more than half of their size, but significantly less compared to the ones covered with membranes.

The histopathologic findings in our experiment demonstrated that the use of membranes as an interface between soft tissue elements and bone was able to prevent the ingrowth of soft tissue into the new bone, *i.e.* in the space between the bone and membrane, contributing therefore to a better filling of mandibular defects with newly formed bone.

The use of human 200 μ thick membrane, as shown in our study, produced better results than the use of human 100 μ thick membrane, since it showed better results in four properties two months after surgery, in two properties four months after surgery and in three properties six months after surgery, especially contributed to maturation of connective tissue and more rapid defect filling with newly formed bone.

In the literature, the papers dealing with the impact of thickness of resorbable membranes on bone regeneration have been scarce. An attempt at using thicker membranes was published in 2005 by Busenlechner et al. [20]. The purpose of their study was to examine the potential of a slowly resorbable, prototype trilayer membrane in bone regeneration for alveolar ridge augmentation after the extraction of the first and the second molars in the monkey mandible, and after cavity formation three months after extraction. The animals were sacrificed after 9 months. The study supported the use of slowly resorbable trilayer membrane, since best bone regeneration was achieved with such a membrane and a bone graft. The membrane was created by the addition of a polylactide layer between two layers of collagen in order to prolong the period of membrane degradation and its barrier function. The fragments of polylactide were found on histologic examination even after 9 months. The design of the trilayer membrane can be an important step towards the improvement of stability of a membrane with a certain degree of exposure. In this study the exposure was 8.33%, which was extremely low compared to 43.75% in the study by Sculean et al. [21] in 1999.

The same prototype of a trilayer membrane was studied by von Arx et al. [22] even before the authors mentioned above (in 2002). Their study tried to examine the prototype of a trilayer membrane in combination with different augmentation materials. The study was done on dogs; after the extraction of premolars, defects were made, into which different augmentation materials and membranes were placed. The dogs were sacrificed after four and a half months, and the sections prepared were histopathologically and histomorphometrically analyzed. The best bone regeneration was demonstrated for the prototype trilayer membrane combined with autograft. In spite of these satisfactory results, the authors could not recommend the membrane for clinical use.

In 2009, Kozlovsky et al. [23] histologically compared the biodegradation of Bio-gide membrane placed in one and two layers into the mechanically made defects on the rat calvarias. The results of the study showed that the percentage of membrane degradation was similar in both cases, but markedly more barrier material remained in the tissue even after 9 months, thus suggesting a longer barrier role of the membrane. Single layer membrane could not perform its barrier function for longer period of time. Bilayer membrane therefore performed better in terms of bone regeneration and defect ossification. It should be pointed out that with the second layer micromovements could be reduced, which in turn significantly improve membrane stability.

In a study by Korean authors, the efficacy of bilayer membrane was analyzed with the use of bone grafts in terms of bone resorption. The study was done in rabbits. Blocks of parietal bone were taken from one side, placed on the contralateral side, and covered with membranes. Histologic and histomorphometric analysis was done 2, 4, and 6 months after surgery. The results of the study showed that the use of bilayer membrane reduced graft bone resorption significantly more compared to singe layer membrane [24].

The results of these few studies examining the membrane thickness, showed that thicker membranes, designed as single- or multilayer structures, demonstrated better barrier performances, maintained longer their presence in the tissue due to their slower biodegradation, and promote better ossification of bone defects, as confirmed by our study as well, since the human resorbable 200 μ thick membrane demonstrated superior regeneration of bone defects.

Conclusion

Based on the results obtained in our study, the resorbable human demineralized 200 μ thick membrane produced a higher degree of bone regeneration compared to resorbable human demineralized 100 μ thick membrane. The use of human demineralized membranes as an interface between bone defects and mucoperiosteal flap soft tissues improved bone regeneration. Bone defects covered with barrier membranes showed better bone healing, although bone regeneration was not complete even after 6 months.

Future studies should investigate the membrane thickness or collagen amounts sufficient to maintain the barrier function beyond 6 months, which is considered the mimimal period of time for guided bone regeneration.

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Received on December 30, 2011.
Accepted on February 14, 2012.

GENERAL REVIEW

UDC: 612.1.062:612.8.067]::616.1-07
DOI: 10.2298/VSP110707019B

Methodology of monitoring cardiovascular regulation

Metodologija praćenja kardiovaskularne regulacije

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Key words:
cardiovascular system; monitoring, physiologic; methods; blood pressure; heart rate; hemodynamics.

Ključne reči:
kardiovaskularni sistem; fiziološke funkcije, praćenje; metode; krvni pritisak; srce, frekvencija; hemodinamika.

Introduction

Adaptation of an organism to changes in external and internal environments is regulated by the autonomic nervous system (ANS) [1–4]. The ANS is structurally and functionally positioned to interface between the internal and external milieu, coordinating bodily functions to ensure normal homeostasis and adaptive responses to environmental changes [4]. The neural control of cardiovascular (CV) system plays a major role in such adaptations, even if different humoral mechanisms also participate in this control. In fact, dynamic environmental changes contrasting basic functional needs of the organism dramatically challenge the CV adaptive mechanisms. The fact that "cardiovascular diseases are the leading cause of death in the world today and will remain so by the year 2020" (The WHO MONICA Project [5]) strongly supports the need for new insights into CV regulatory mechanisms. This review considers recent studies which focus on the understanding of CV regulation and the methodology for monitoring CV regulation.

Cardiovascular regulation

CV neural regulation occurs through both sympathetic and parasympathetic (vagal) outflow to the heart and vessels. Central autonomic drives act directly from the central nervous system (CNS) on the heart and vessels, while peripheral drives are relayed to the heart and vessels through, among others, the baroreflex function. These drives are relayed to the heart through sympathetic and parasympathetic outflows and to blood vessels through sympathetic outflow only (Figure 1). Classically, central integration modifies the performance of individual reflexes according to the prevailing behavioural needs (*ie* exercise) [6]. Thus, heart period (HP) is modified together with other controlled hemodynamic variables, such as vascular resistance and, consequently, arterial blood pressure (BP) [7]. Numerous techniques for studying autonomic control of CV system are based on HP and arterial BP analysis (Figure 2). These techniques can be divided in two groups: techniques based on induced fluctuations of arterial BP and techniques based on analysis of spontaneous fluctuations of BP (Figure 2). Here we put emphasis on the techniques of HP and BP spontaneous fluctuations due to their numerous advantages with respect to the techniques based on induced fluctuations of arterial BP. The resulting HP and arterial BP values obtained by this technique reflect the overall interaction between central and peripheral mechanisms of CV regulation, without providing straightforward information on separate central and peripheral contributions being the result of rather complex interplay [8]. As a result of the activity of different mechanisms involved, HP and arterial BP fluctuations are commonly observed in physiological conditions. These fluctuations are present even in the absence of motor behaviour, like in paralysed animals [9]. Such fluctuations in hemodynamic parameters reflect both the presence of a variety of naturally occurring physiological perturbations to CV homeostasis (i.e. respiration, postural shifts, thermoregulation) and the dynamic response

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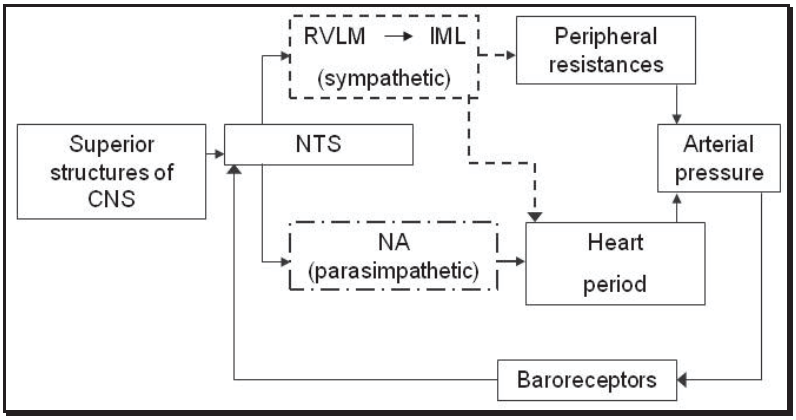

Fig. 1 – Mechanisms of peripheral (baroreflex) and central regulation of cardiovascular system
NTS – *nucleus tractus solitarii;* RVLM – rostral ventrolateral medulla; IML – intermediolateral column; NA – *nucleus ambiguus*.
Long dash spotted line-parasympathetic nervous system drive, dashed line-sympathetic nervous system drive

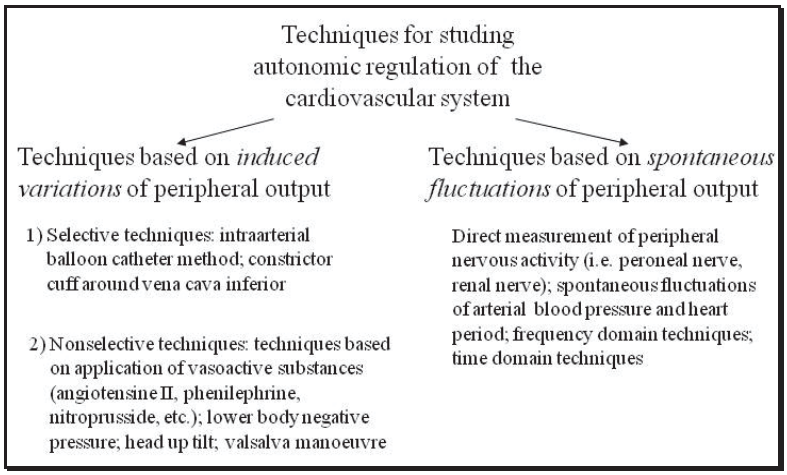

Fig. 2 – Scheme of techniques for studying the autonomic regulation of cardiovascular system

of the CV control systems to these perturbations [10]. Increase of arterial BP and HP variability after sinoaortic baroreceptor deafferentation and their decrease after following pharmacological sympathetic and parasympathetic ganglionic block [9, 11] suggest that a significant part of spontaneous, steady-state arterial BP and HP variability is due to the interaction in central and peripheral mechanisms of CV control.

Central control

There is a lack of data on the central pathways subserving "central command" responses (Figure 1). The idea of a "central command" signal originated from the observations that heart rate, ventilation and BP increase almost immediately at the onset of voluntary exercise [6]. "Central command", in the case of BP and heart rate increase at the beginning of the exercise has a clear functional implication in matching blood flow to increased metabolic rate of an organism [12].

A dramatic demonstration of "central command" as a feed-forward regulation in the absence of muscle activity is shown in the study of Gandevia et al. [13], in which a paralysed, artificially ventilated human subject attempted to perform isometric contractions. It resulted in marked concomitant increases in arterial BP and heart rate, which were graded according to the degree of the attempted force. Concomitant hypotensive and bradycardic changes central by origin are found in sleep [14] and opioid anesthesia [15]. According to our findings central command has different impact on the organism with respect to the age [16].

A number of cerebral areas appear to be involved in central control of CV function. These areas are mostly located in the frontal cortex and include parts of the cingulate and insular cortex [17], orbitofrontal cortex [18], amygdala [19], dorsomedial hypothalamic nucleus [20] and midbrain [21, 22].

Baroreflex control

Baroreflexes represent classic negative feed-back mechanisms (Figure 1). Changes in baroreceptor input to the brain provoke changes in neural output in two branches of the ANS – sympathetic and parasympathetic branches. The parasympathetic (vagal) system controls about 75% of the fastest baroreflex effector loop – heart rate, up to 100 beats/min. The sympathetic system controls the remaining 25% of this effector, and further controls conductance and contractility of the heart, and total peripheral resistance [1].

The parasympathetic system acts fast and powerfully, and can change heart rate within one beat [1]. Due to the different dynamics of neurotransmitter release, different intra-

cellular effector molecular mechanisms and different mechanisms of neurotransmitter removal from neuromuscular synaptic cleft, sympathetic nervous system act slower with respect to parasympathetic system [23].

Blood pressure set point

Baroreceptor reflex performance is modified by various mechanisms of the CNS. Though criticised for erroneous associations it might provoke between biological systems and the servo control system, the term "set point" is in use for description of the "desired level" of BP and baroreflex mechanism [24].

The set point (level around which arterial pressure is regulated) varies under different physiological (*ie* exercise) [6] and pathophysiological (*ie* hypertension) [25] conditions. A natural selection appears to favour a control system that regulates arterial pressure around a set point that varies according to an animal's behaviour [24].

Baroreflex sensitivity

Baroreflex sensitivity (BRS), or baroreflex gain is defined as a transfer function between a primary (input) change in BP and reflex (output) compensatory change in BP or heart rate [24]. From the classical studies of baroreflex functioning [26] till the recent investigations [9, 11 27–29] the open loop baroreflex studies were done in anesthetised or pharmacologically treated animals. In conscious animals and humans, it is very difficult to perform an open-loop analysis of baroreflex gain.

Methods for studing mechanisms of cardiovascular regulation-techniques

Techniques for analysis of HP and BP induced fluctuations

These methods apply external stimulus for the evaluation of baroreflex loop and perform so-called "spot" analysis of BRS [30].

Many basic laboratory techniques are applicable to experimental evaluation of selective carotide or aortic arch baroreceptors (intravascular occlusion of corresponding artery [31]. Selective unloading of cardiopulmonary baroreceptors is performed by an inflating cuff placed around *vena cava inferior*. All these methods are invasive, demanding anesthesia and applicable only to laboratory animals.

Non-selective tests of baroreceptor function stimulate whole groups of baroreceptors, without any care for their regional and functional differences.

Orthostatic tests like stand test, tilt test and lower body negative pressure (LBNP) test are widely used for clinical and scientific purposes of investigating CV regulation [32]. The head-up tilt test is the method used for investigation of syncope, presyncope, dizziness and palpitations related to dysautonomia symptoms [33]. Lately, the test has been criticized due to great variation in sensitivity and specificity rates in different studies, as well as for its limited accuracy and reproducibility [34]. In this technique baroreflex stimulus is physiological, but the specificity is limited, due to unloading of cardiopulmonary baroreceptors and stimulation of vestibular centers [35].

LBNP induces, with the depression below iliac crest, fluid shift (blood and interstitial fluids) towards the lower part of the body. LBNP stimulates CV system, in a particular a baroreflex regulation loop by unselective unload of these receptors. The LBNP test can cause syncope and progressive fluid shift can cause CV changes that are not stationary, not providing this important condition for further mathematical analysis of the signal [36].

A method for application of vasoactive substances (Oxford method) was founded by Smyth et al., [37] in 1969. It is based on intravascular injection of vasoactive substances, like angiotensin II, phenylephrine or nitroprusside. It is used as the gold standard method for BRS measuring. It is methodologically simple, more specific, but it quantifies only arterial BP-HP baroreflex loop. Vasoactive substances also act directly on the CNS structures, cardiopulmonary receptors, as well as on sinoatrial node [38].

Valsalva manoeuvre is based on tachicardic or bradicardic response on the initial decrease or increase of arterial BP appearing during constant expiratory pressure (40 mmHg) lasting for 15–20 s. It is a noninvasive, simple method, but its disadvantages are the involvement of chemoreceptors, cardiopulmonary baroreceptors, muscle receptors and it also requires active collaboration of a patient [39].

Techniques for analysis of HP and BP spontaneous fluctuations

A basic methodological advantage of these techniques is continuous measurement of BRS and higher level of sensitivity on baroreflex disfunction as compared to classical methods [30, 40, 41].

Direct measurement of sympathetic nerve activity in peroneal nerve or in renal nerve allows measurement of BRS as the responsiveness of sympathetic nervous activity to the changes of BP [42]. The sympathetic bursts are synchronized with transient reductions of BP and are silenced during increased pressure [43].

Spontaneous fluctuations in HP and arterial BP have been explored both in frequency and time domains during the last two decades. Spontaneous sequences of HP and arterial BP beat-to-beat values have been used to study different aspects of CV regulation in physiological and pathophysiological states. An important step in evaluation of CV control came from the recognition that oscillations in HP and BP result both from the operation of feed-back regulatory loops [41, 44] and from "central commands" [45–47]. In clinical use, there are different software packages, like Nevrokard®, compatible with Finapress®, Portapress®, Colin® and BIOPAC® monitors.

Frequency domain techniques

Studies on the frequency domain [10, 40, 48, 49] have provided a novel insight into the interplay of sympathetic and vagal CV modulations, leading to new tools for studying CV control. The frequency components of these fluctuations can

be assessed by spectral analysis [50] and reflect major changes in autonomic control of heart and vessels. HP power spectra depict the modulation of autonomic control on sinoatrial node, not its absolute value [51]. In many conditions, the modulation amplitude is proportional to its absolute value [48, 52]. In HP power spectra, the low-frequency band (less than 0.15 Hz in humans [50], 0.45 Hz in rats [53], 0.6 Hz in mice [54]), has been associated with the modulation of both sympathetic and parasympathetic outflow, while the high frequency band (greater than 0.15 Hz in humans [50], 1.04 Hz in rats [53], 1.0 Hz in mice [54]) has been associated with the modulation of parasympathetic outflow [55]. The contribution of sympathetic and parasympathetic efferent activity to low frequency and high frequency HP and BP power spectra, respectively, has been confirmed during wakefulness [56] and sleep [57] by experiments using selective pharmacological blockade (propranolol, atropine).

Time domain techniques

Analysis of the continuous relationship of beat-by-beat changes in arterial pressure and HP revealed that spontaneous increases or decreases in systolic arterial pressure ("ramps") induce directionally similar reflex changes in HP [58]. On this basis, a novel technique called "spontaneous baroreflex analysis" was developed for dynamic studying of the arterial baroreflex control of the sinus node [44]. This widely accepted method [45, 59–62] is based on a computer scanning of BP and HP time series to identify sequences of spontaneously occurring consecutive beats in which BP and HP change in the same direction, (named "baroreflex sequences") i.e. hypertensive/bradycardic and hypotensive / tachycardic sequences [44].

Measuring BRS from spontaneous variations in BP and heart rate [44] has several advantages over methods that artificially induce changes in BP. This method excludes administration of vasoactive compounds or external appliances that could influence the baroreceptor reflex by a direct action on receptor or effector sites [63]. BRS is measured within physiological BP ranges, allowing computation of the gain at BP close to the operating set point value, with minimal non-specific effects from other efferent nerves [40]. The baroreceptor gain thus obtained is closest to the physiological one. These methods do not arouse subjects or animals, thereby reducing stress-induced effects. In contrast to pharmacological or mechanical methods, they are suitable to assess BRS over prolonged periods of time [30, 41].

Methods that evaluate BRS from spontaneous changes in BP and HP make use of linear regression analysis of HP *vs* spontaneously occurring ramps in BP [44, 58, 64], and of spectral analysis [65] or other statistical relationships between BP and pulse interval changes [66].

BRS calculated as a slope of HP *vs* BP linear regression in spontaneously occurring pressure ramps [44] shows the best correlation to reference pharmacological methods and gives zero value following interruption of the baroreflex arch [41, 44, 60].

Apart from "baroreflex sequences", beat-to-beat analysis of the continuous relationship between spontaneous fluctuations in BP and HP also reveals the occurrence of sequences of consecutive beats in which BP and HP change in the opposite direction (*ie* hypertensive/tachycardic and hypotensive/bradycardic sequences). These sequences have been defined as "non-baroreflex" [44].

The physiological meaning and thus the possible role of non-baroreflex sequences in evaluation of central command of the CV regulation is still controversial. Oosting et al. [41,] include in BRS index calculation all BP sequences, non-baroreflex sequences as well, regardless the direction of HP changes with respect to pressure changes. The idea behind this approach is that the relationship between HP and arterial BP includes both baroreflex and random influences; if baroreflex – mediated effects on HP are present, they should appear as such when averaging over ramps is performed [41]. In addition, this technique included $49.8 \pm 4.1\%$ of all the recorded beats in BRS index calculation. Calculation also included a significant number of sequences that corresponded to non-baroreflex ones.

The main limitation of this approach is that the BRS index is mainly a measure of parasympathetic reaction, being calculated on short sequences (9.7 ± 1.6 beats, mean ± SEM) and with a delay of HP *vs* arterial BP (3, 4 and 5 beats) that is too short to take account a full sympathetic reaction to an arterial pressure change [46]. HP changes induced by vagal reactions would superimpose upon slow sympathetically induced ones [41].

Furthermore, Legramante et al. demonstrated in anesthetised rabbits [62] and humans [45] that spontaneously occurring non-baroreflex sequences can be considered an expression of autonomic regulatory mechanisms operating with feed-forward features, as it is the case of "central command". They have calculated a baroreflex gain on sequences where heart rate and BP changed in the same direction [44], while the gain of feed-forward mechanisms was calculated on non-baroreflex sequences. The same authors demonstrated that both branches of the ANS take part in feed-forward mechanisms of short-term CV neural regulation [45, 62]. Recent investigation on conscious freely moving rats [47] have provided further evidence that non-baroreflex sequences reflect mechanisms feed forward in origin. A complete autonomic pharmacological blockade reduced the number of non-baroreflex sequences, as did sympathetic blockade, selective alpha-receptor blockade did not induce changes, while beta-receptor blockade induced a significant decrease in non-baroreflex sequences occurrence. Moreover, parasympathetic blockade induced increase in non-baroreflex sequences. The results of Pagani et al. [48] demonstrate that physiological role of non-baroreflex sequences is an expression of feed-forward type of short term CV regulation being in dynamic interaction with feed-back mechanisms of baroreflex origin.

Still, the intrinsic limitation of this method in evaluating feed-forward mechanisms is a small number of beats (in animals ≈5% [62] and in humans ≈7% [45]) organised in sequences characterised by a non-baroreflex pattern. This finding is in contrast with the fact that feed-forward mechanisms can be engaged for a prominent fraction of time [6].

Zoccoli et al. [46] suggest that parallel analysis of both the BRS index of Bertinieri et al. [44] and the BRS index of Oosting et al. [41], and a novel index in time domain sensitive to slow sympathetic fluctuations would overcome: the limitations of the method of Oosting et al. [41] in estimating the relative contribution of feed-back control, and the limitations of the method of Legramante et al. [47] in estimating feed-forward control over HP and offer a more complete picture of the interrelation between peripheral and central mechanisms in HP control. The index [46] b_{HPMAP} is calculated as an index of linear regression of arterial BP *vs* HP 30s sequences. It correlates well with indexes of Bertinieri et al. [44] and Oosting et al. [41] in quiet wakefulness of the conscious rats, while in active sleep correlates significantly with the sympathovagal index. We have reported that the index b_{HPMAP} can reflect sympathetic changes in the time domain as well [46]. This data suggest that the overall picture of baroreflex-central command interaction can be achieved by comparative analysis of more than one method for calculation of BRS and feed-forward gain proposed in the literature.

Short-comment and conclusion

It is well-known that phasic and tonic increases in central drive to the heart both as impaired baroreflex regulation might increase the incidence and severity of cardiac arrhythmias [67]. An increased central drive is also present in acute stress (classic "defense" or "alerting" response [68], chronic psychological stress [69], acute physical stress [70] as well as during arousal [71], acoustic stimulation [72]). In all circumstances, the central drive and impaired baroreflex both were positively correlated to the incidence of cardiac arrhythmia in susceptible subjects. On the basis of these results, the techniques of CV monitoring keep an important place in studying pathophysiological mechanisms of arrhythmogenesis.

Acknowledgement

This work was supported by grants No. 173033 (to Esma R. Isenović) and III 41028 (Dragan Alavantić) from the Ministry of Science, Republic of Serbia. We would like to thank Prof. Carlo Franzini for his contribution in writing this text.

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Received on July 7, 2011.
Revised on September 5, 2011.
Accepted on October 11, 2011.
OnLine-first, June, 2012.

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

UDC: 575:[616.9-085:615.281.828
DOI: 10.2298/VSP1212091D

Farmakogenetika antiretrovirusnih lekova

Pharmacogenetics of antiretroviral drugs

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Ključne reči:
hiv; antiretrovirusni lekovi; lečenje, ishod; farmakogenetika; polimorfizam, genetički.

Key words:
hiv; anti-retroviral agents; treatment outcome; pharmacogenetics; polymorphism, genetic.

Uvod

Sindrom stečene imunodeficijencije (na francuskom *syndrome d'immuno-deficence acquis* – SIDA ili na engleskom *acquired immunodeficiency syndrome* – AIDS) predstavlja terminalni stadijum hronične infekcije izazvane virusom humane imunodeficijencije (*human immunodeficiency virus* – HIV).

Osvrtom na hronologiju višedecenijske borbe protiv AIDS-a, prepoznaju se fascinantni rezultati bazičnih biomedicinskih nauka u lečenju ove infekcije. Opsežna virusološka, imunološka, farmakološka i klinička istraživanja, brže i efikasnije nego ikada ranije u istoriji farmakoterapije, dovela su do sinteze antiretrovirusnih lekova.

Hronološki posmatrano, 1981. godine opisani su prvi klinički slučajevi do tada nepoznate bolesti, a već 1983. godine Barre-Sinoussi i sar. [1] izolovali su „novi“ virus nazvan T-limfotropni humani retrovirus (*lymphadenopathy associated virus* – LAV), danas poznat pod nazivom HIV, koji je prepoznat kao izazivač AIDS-a. Od tada je trebalo da prođu četiri godine do sinteze prvog [zidovudin (AZT)] i još celih pet godina do sinteze drugog, [didanozin (ddl)] antiretrovirusnog leka. Primena pomenutih antiretrovirusnih lekova u vidu monoterapije vrlo brzo se pokazala nedovoljno efikasnom i neadekvatnom zbog kratkotrajnog kliničkog poboljšanja i visoke stope morbiditeta i mortaliteta od AIDS-a [2]. Tek od 1996. godine blesnula je „nada spasa“, sa uvođenjem kombinovane visokoaktivne antiretrovirusne terapije (*highly active antiretroviral therapy* – HAART), odnosno kombinacije 3–4 antiretrovirusna leka različitog mehanizma dejstva [3].

Danas HAART najčešće čine kombinacije dva leka iz grupe nukleozidnih inhibitora reverzne transkriptaze (NIRT) sa jednim lekom iz grupe proteaznih inhibitora (PI) – 2 NIRT+1 PI. Međutim, podjednako je efikasna i kombinacija dva leka iz grupe NIRT sa jednim nenukleozidnim inhibitorom reverzne transkriptaze (NNIRT) – 2 NIRT+1 NNIRT, na koji način se PI „štede“ za kasnije faze lečenja. Primenom HAART-a postiže se maksimalna i dugotrajna supresija replikacije HIV-a, što dovodi do značajnog usporavanja progresije bolesti, boljeg kvaliteta života i, što je najvažnije, do produženja životnog veka bolesnika sa HIV infekcijom [4].

U poslednjih nekoliko godina primenjuje se nova strategija „pojačavanja“ dejstva PI sa ciljem da se poveća njihova terapijska efikasnost. Strategija „pojačavanja“ podrazumeva modifikaciju farmakokinetike PI dodavanjem malih doza (100 mg/dan) ritonavira (RTV) koji inhibiše enzim citohrom P450 3A (CYP3A) u jetri. Na ovaj način značajno se produžava poluživot svih PI, pa se danas, prema važećim smernicama za racionalnu antiretrovirusnu terapiju, RTV koristi isključivo za „pojačavanje“ dejstva ostalih lekova iz grupe PI. Zbog istovremene primene sa RTV, proteazni inhibitori mogu se primenjivati dva puta ili čak jednom dnevno, što olakšava njihovu svakodnevnu primenu [5, 6].

Među savremenim protokolima kombinovane primene antiretrovirusne terapije postoji i tzv. terapija „spasavanja“ (*salvage*) koja podrazumeva primenu svih poznatih klasa lekova u situacijama kada sve prethodne terapijske opcije nisu bile efikasne [4].

Za sada je u svetu registrovano ukupno 25 antiretrovirusnih lekova, klasifikovanih u pet grupa koje deluju na različitim nivoima replikacionog ciklusa HIV-a:

1. NIRT: AZT, zalcitabin, ddl, stavudin, lamivudin, abakavir, emtricitabin i tenofovir.
2. NNIRT: nevirapin, delavirdin, efavirenz i etravirin.
3. PI: sakvinavir, RTV, indinavir, nelfinavir, amprenavir, lopinavir, fosamprenavir, atazanavir, tipranavir i darunavir.
4. Inhibitori fuzije HIV-a: enfuvirtid (klasični inhibitor fuzije) i maravirok (antagonista CCR5 receptora).
5. Inhibitori virusne integraze: reltegravir.

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Doživotna terapija jednom kombinacijom različitih antiretrovirusnih lekova nije moguća, pre svega zbog toksičnosti i razvoja rezistencije HIV-a na antiretrovirusne lekove, pa kod mnogih bolesnika „magija“ HAART-a više „ne deluje“. Pad mortaliteta i morbiditeta od AIDS-a poslednjih godina dostigao je plato koji se i dalje održava, ali i nagoveštava da je i najoptimističkiji scenario o efikasnosti HAART-a još uvek daleko od težnje bolesnika i njihovih lekara da se sa HIV-om „normalno“ živi [2].

Terapijska efikasnost i interindividualni odgovor na propisanu visoko aktivnu antiretrovirusnu terapiju

Iako je primena HAART-a dovela do značajnog sniženja morbiditeta i mortaliteta, dosadašnja iskustva otkrivaju i njene nedostatke. Neophodno višegodišnje svakodnevno uzimanje velikog broja različitih lekova sa širokim spektrom neželjenih efekata, pri čemu neki od njih mogu imati fatalan ishod, uz pojavu neželjenih interakcija sa drugim lekovima i hranom, čine ovaj koncept lečenja vrlo komplikovanim za bolesnika, ponekad sa negativnim uticajem na kvalitet života. Pored toga, vremenom se razvija rezistencija virusa na pojedine klase antiretrovirusnih lekova, što dodatno otežava i sužava izbor lekova za naredne kombinacije. Kao posledica velike mutagene sposobnosti HIV-a, u prisustvu leka ili pojedinih lekova u plazmi dolazi do selekcije rezistentnog kvazisoja HIV-a koji, potom, može i da prevlada. Akumulacija mutacija dovodi ne samo do rezistencije na jedan lek, već i do unakrsne rezistencije na većinu ili čak na sve lekove istog mehanizma dejstva [7–9].

I pored poznavanja svih faktora važnih za postizanje uspešnog terapijskog ishoda, sve češće smo suočeni sa činjenicom da je HAART neuspešan kod određenog broja bolesnika koji redovno i pravilno uzimaju konvencionalne terapijske doze, a pritom nemaju problem rezistencije virusa na lekove [10]. Istovremeno, kod nekih bolesnika sa dobrim terapijskim odgovorom na HAART registruju se teški neželjeni efekti koji se ne javljaju kod drugih bolesnika koji koriste iste doze istih antiretrovirusnih lekova [11]. Zbog ovih problema otvorila se mogućnost da farmakokinetska praćenja (istraživanja) HAART-a postanu deo rutinske kliničke prakse [12]. Rezultati farmakokinetskih istraživanja ukazuju na ogromne interindividualne varijacije koncentracija antiretrovirusnih lekova u plazmi kod primene standardnih, preporučenih dnevnih doza ovih lekova. Farmakokinetska varijabilnost utiče ne samo na terapijski ishod, već i na pojavu neželjenih i toksičnih efekata antiretrovirusnih lekova [10, 12].

Zasada je utvrđeno da na farmakokinetske faktore mogu da utiču individualne karakteristike samog bolesnika [pol, godine starosti, telesna masa, indeks telesne mase (BMI), trudnoća, rasna i etnička pripadnost, način ishrane, konzumiranje alkohola i narkotika, očuvanost funkcije kardiovaskularnog, hepatičkog, renalnog i gastrointestinalnog sistema], kao i prisustvo istovremenih infekcija [hepatitis C virusna (HCV) infekcija, hepatitis B virusna (HBV) infekcija, kao i infekcija izazvana *Mycobacterium tuberculosis*] [13]. I sama genetička struktura bolesnika, prevashodno polimorfizmi gena odgovornih za metabolizam i transport antiretrovirusnih lekova, takođe, mogu da utiču na interindividualne varijacije u terapijskom odgovoru na propisani HAART [14].

Uticaj genetičkih polimorfizama na terapijsku efikasnost visokoaktivne antiretrovirusne terapije

Interesovanje za uticaj genetičkih polimorfizama na terapijsku efikasnost različitih grupa lekova počelo je da raste sredinom devedesetih godina prošlog veka. Naime, već 1990. godine započet je projekat pod nazivom *Human genome project*, čiji cilj je bio da se do 2005. godine izvrši mapiranje humanog genoma radi boljeg razumevanja genetičkih faktora u nastanku i razvoju pojedinih bolesti. Istraživanja podstaknuta mapiranjem humanog genoma ubrzala su otkrića genetičkih varijacija koje utiču na terapiju određenih bolesti, kao i razvoj novih lekova. Zahvaljujući dostignućima biotehnologije rad na mapiranju humanog genoma završen je znatno pre postavljenog roka [15].

Genetički polimorfizmi su izraz normalne raznolikosti u naslednoj osnovi čoveka. Postoje tri osnovna tipa genetičkih polimorfizama: pojedinačni nukleotidni polimorfizmi (PNP), polimorfizmi broja uzastopnih ponovaka i deleciono/insercioni polimorfizmi. Od svih navedenih, najčešći polimorfizmi su PNP koji predstavljaju varijacije pojedinačnih nukleotida, odnosno adenina (A), timina (T), citozina (C) i gvanina (G) na specifičnom lokusu među parovima homologih hromozoma jedinki iste vrste [15]. Kada se dva sekvencirana fragmenta DNK različitih individua iste vrste razlikuju samo za jedan nukleotid (npr. AAGCCTA→AAGCTTA), tada se govori o PNP i smatra se da postoje dva tipa alela: tip C i tip T [15].

U poslednjih nekoliko godina rezultati farmakogenetičkih istraživanja ukazuju na to da postojanje PNP ima značajan uticaj na terapijsku efikasnost antiretrovirusnih lekova. Pokazano je da na terapijsku efikasnost antiretrovirusnih lekova mogu da utiču polimorfizmi gena koji su odgovorni za njihov metabolizam i transport [16–18].

Pojedinačni nukleotidni polimorfizmi gena odgovornih za sintezu enzima uključenih u metabolizam antiretrovirusnih lekova

U humanoj populaciji citohrom P450 ima ključnu ulogu u biotransformaciji velikog broja lekova, uključujući i antiretrovirusne lekove. Do sada je kod čoveka otkriveno 57 različitih CYP izoenzima, od čega su za metabolizam antiretrovirusnih lekova važni: CYP3A4, 3A5, 2B6 i manje važni: 2C9, 2C19, 2D6 i 2E1 [19, 20].

Pojedinačni nukleotidni polimorfizmi gena koji kodiraju sintezu CYP450 enzima mogu da dovedu do promene funkcije ovog enzimskog sistema. Izmenjena funkcija CYP450 posledično utiče na varijabilnost koncentracije antiretrovirusnih lekova u plazmi, što dalje utiče na terapijski odgovor i pojavu neželjenih efekata ovih lekova [21, 22].

Polimorfizam gena CYP3A4

Izoenzim CYP3A4 predstavlja glavnu izoformu familije CYP3A, koja je od svih humanih izoenzima CYP najviše ispitana do sada. Proenzim CYP3A4 metaboliše više od 50% svih postojećih grupa lekova, uključujući i antiretrovirusne lekove, kao što su delavirdin, sakvinavir, indinavir, RTV, nelfinavir, i drugi. Interindividualna varijabilnost u ekspresiji

enzima CYP3A4 vrlo je velika, što dovodi do interindividualnih razlika u dispoziciji lekova koji su supstrat CYP3A4 [23].

Sintezu enzima CYP3A4 kodira istoimeni gen, lociran na hromozomu 7q21, koji se sastoji od trinaest egzona. Unutar gena za CYP3A4 opisano je dvadesetak mutiranih alela koji se u populaciji belaca pojavljuju s učestalošcu do 7%; međutim, tek je za manji broj potvrđeno da modifikuju funkciju enzima. Najznačajniji i zasada najbolje proučen polimorfizam u genu CYP3A4 je polimorfizam A > G na poziciji -392, na kojoj se umesto adenina (A) može naći guanin (G). Kada je na poziciji -392 na genu CYP3A4 A, tada govorimo o normalnom (tj. nemutiranom) obliku gena, a ukoliko je na istoj poziciji G, tada je reč o mutiranom obliku gena. Ovaj polimorfizam je u regionu 5'UTR gena CYP3A4, nekodirajućoj promotorskoj regiji. On se označava kao CYP3A4*1B i povezan je sa pojačanom aktivnošću enzima CYP3A4 [23].

Farmakogenetička istraživanja ukazuju na to da je PNP direktna posledica ne samo rasne, već i etničke pripadnosti. Tako je u populaciji Afroamerikanaca („Black Americans“) prisutan mutirani oblik gena CYP3A4, kao najčešći oblik, usled čega se smanjuje klirens pojedinih antiretrovirusnih lekova [24].

U tabeli 1 navedeni su geni CYP, PNP geni odgovorni za metabolizam i njihov uticaj na farmakokinetiku, odnosno farmakodinamiku antiretrovirusnih lekova.

ispituje. Do sada je nađen veliki broj različitih alela gena CYP3A5, ali su mnoge otkrivene mutacije nefunkcionalne. Samo su retki polimorfizmi funkcionalni, odnosno imaju značajan uticaj na funkciju izoenzima CYP 3A5, kao što je PNP na poziciji 6986 u genu CYP 3A5 [23]. Pomenuti PNP utiče na pojavu povišenih vrednosti atazanavira u plazmi i posledičnu pojavu hiperbilirubinemije i žutice. Isti PNP takođe utiče na povišenje vrednosti efavirenza u plazmi i pojavu neželjenih efekata od strane centralnog nervnog sistema (CNS) (tabela 1).

Rezultati farmakogenetičkih studija međutim, i dalje su prilično oprečni što se tiče mesta i uloge polimorfnih gena CYP3A5 u metabolizmu antiretrovirusnih lekova, kao i učestalosti PNP među bolesnicima, pripadnicima različitih rasnih grupa [30].

Polimorfizam gena CYP 2B6

Sintezu izoenzima CYP2B6 kodira istoimeni gen lociran na hromozomu 19. Pojedinačni nukleotidni polimorfizmi koji imaju značajan uticaj na farmakokinetiku antiretrovirsnih lekova identifikovani su na egzonu 4 na poziciji 516 i na egzonu 9 na poziciji 1459T [23]. Tako na poziciji 516 umesto guanina (G) može se naći timin (T) u genu CYP2B6. Kada je na poziciji 516 u genu CYP2B6 gvanin (G), tada govorimo o normalnom (tj. nemutiranom) obliku gena. Ukoliko je na istoj poziciji timin (T), tada je reč o mutiranom obliku gena [22]. Kada je reč o PNP na poziciji 1459 na genu CYP2B6, utvrđeno je da normalni (tj. nemutirani) oblik gena CYP2B6 ima citozin (C) na poziciji 1459, dok mutirani oblik gena ima timin (T) na istoj poziciji [23].

Tabela 1

Geni citohroma P450 (CYP), i pojedinačni nukleotidni polimorfizmi (PNP) gena odgovornih za metabolizam i njihov uticaj na farmakokinetiku, odnosno farmakodinamiku antiretrovirusnih lekova

Gen	PNP	Lek	Farmakokinetski efekat	Framakodinamski efekat
CYP2B6	CYP2B6*26 (516G>T)	efavirenz	visoke koncentracije efavirenza u plazmi (preporuka: redukcija doze kod nosilaca CYP2B6*6/*6 i *6/*26)	u direktnoj vezi sa neuropsihijatrijskim komplikacijama; poboljšanje simptoma od strane CNS-a posle redukcije doze [25]
CYP2B6	CYP2B6*5 (1459C>T)	efavirenz	nejasan	nejasan [26]
CYP2B6	CYP2B6*5 (1459C>T)	nelfinavir	nejasan	nejasan
CYP2B6	CYP2B6*6 (516G>T)	nevirapin	nosioci ovog alela imaju povišene vrednosti nevirapina u plazmi	Porast broja CD4 ćelija i oporavak imunskog sistema [26–28]
CYP3A4	CYP3A4*1B (-392A>G)	efavirenz	nejasan	nejasan [29]
CYP3A4	CYP3A4*1B (-392A>G)	indinavir	snižene vrednosti u plazmi	nejasan [29]
CYP3A4	CYP3A4*1B (-392A>G)	nelfinavir	nepoznat	nejasan [29]
CYP3A5	CYP3A5*3 (6986A>G)	atazanavir	povišene vrednosti u plazmi	žutica i hiperbilirubinemija [30]
CYP3A5	CYP3A5*3 (6986A>G)	efavirenz	povišene vrednosti u plazmi	neželjeni efekti CNS-a [30]
CYP3A5	CYP3A5*3 (6986A>G)	indinavir	nepoznat	nejasan
CYP3A5	CYP3A5*3 (6986A>G)	lopinavir/r	nema efekta	nema efekta
CYP3A5	CYP3A5*3 (6986A>G)	nelfinavir	nepoznat	nepoznat
CYP3A5	CYP3A5*3 (6986A>G)	sakvinavir	povišene vrednosti sakvinavira u plazmi	nepoznat

CNS – centralni nervni sistem

Polimorfizam gena CYP3A5

Sintezu CYP3A5 kodira istoimeni gen koji je, takođe, lociran na hromozomu 7q21 [23]. U poslednjih nekoliko godina funkcionalni polimorfizam na genu CYP3A5 intenzivno se

Antiretrovirusni lekovi koji se delom ili u potpunosti metabolišu pod dejstvom CYP2B6 su efavirenz i nevirapin [25]. Interesantno je da aktivnost izoenzima CYP2B6 može da varira i nekoliko stotina puta među različitim individuama [25–27]. Ovaj podatak ukazuje na mogućnost postojanja ogromnih farmakokinetskih varijacija efavirenza i nevirapina kod različitih individua.

I dalje nije u potpunosti poznat uticaj PNP na poziciji 1459 na egzonu 9 na genu CYP2B6 na minimalne koncentracije u plazmi i na terapijsku efikasnost antiretrovirusnih lekova. Za sada je jedino poznato da PNP na poziciji 516 na egzonu 4 dovodi do pojave visokih koncentracija efavirenza u plazmi [27]. Zbog pojave neželjenih efekata CNS preporuka je redukcija doze kod nosilaca CYP2B6*6/*6 i *6/*26 (tabela 1).

Polimorfizmi gena odgovornih za sintezu proteina uključenih u transport antiretrovirusnih lekova

Polimorfizmi gena koji kodiraju sintezu transportnih proteina dovode do različitog stepena njihove ekspresije, što dalje utiče na stepen distribucije lekova, koncentracije ovih lekova u plazmi i njihovu terapijsku efikasnost.

Dva najviše izučavana efluksna transportna proteina su P-glikoprotein (Pgp) i protein odgovoran za razvoj rezistencije na veliki broj lekova (*multidrug resistance-associated protein-1* – MRP-1), koji omogućavaju transport antiretrovirusnih lekova iz grupe NNIRT i PI. Sniženje intracelularne koncentracije antiretrovirusnih lekova iz grupe NNIRT i PI izaziva pojačano izbacivanje, tj. efluks ovih lekova iz ćelije pod dejstvom Pgp i MRP-1 efluksnih pumpi, koje se nalaze na i/ili u ćelijskoj membrani [31, 32].

Polimorfizam gena ABCB1 koji kodira sintezu P-glikoproteina

Transportni proteini imaju važnu ulogu u regulaciji apsorpcije, raspodele i izlučivanja mnogih lekova. Najbolje do sada proučen je Pgp, čiju sintezu kodira gen ABCB1. P-glikoprotein je integralni membranski efluksni protein. Fiziološka uloga ovog transportnog proteina je da zaštiti organizam od različitih citotoksičnih agenasa endogenog i/ili egzogenog porekla. Veliki broj lekova su supstrati za Pgp, stoga aktivnost Pgp-a utiče na njihove farmakokinetske parametre, međusobne interakcije i terapijski efekat. Protein Pgp je prisutan u organima poput creva, placente, bubrega, jetre, pankreasa, testisa, krvnomoždane barijere, limfocita, makrofaga, gde ima ulogu moduliranja bioraspoloživosti leka. Ekspresija Pgp-a u ovim tkivima rezultira sniženom apsorpcijom lekova iz gastrointestinalnog trakta, pojačanom eliminacijom leka putem žuči i putem urina i usporenim ulaskom pojedinih lekova u CNS [31]. Klinički značaj uloge Pgp-a zavisi od njegove lokalizacije, terapijskog indeksa lekova-supstrata i intraindividualne varijabilnosti. S obzirom na varijabilnost, istraživanja polimorfizama gena ABCB1 pokazala su značajnu korelaciju nekih genotipova sa promenama u farmakokinetici i interakcijama klinički važnih lekova poput citostatika, steroida, peptida, blokatora kalcijumskih kanala, antihistaminika, ali i anitretrovirusnih lekova iz grupe NNIRT i PI [23]. Danas je sve veći broj pretkliničkih i kliničkih studija čiji rezultati pokazuju da bi polimorfizam gena ABC1 mogao biti važan pokazatelj u proceni ishoda lečenja velikog broja oboljenja [33, 34].

Gen ABC1 lociran je na hromozomu 7q21. Do sada je opisano više mutacija u genu i istražena njihova povezanost s ekspresijom Pgp-a i uticaj na farmakokinetske parametre mnogih, pa i antiretrovirusnih lekova [31].

Farmakogenetičkim istraživanjima utvrđeno je da kod različitih individua, nosilaca različitih alela u genu za MDR1, dolazi do različite ekspresije efluksne pumpe Pgp-a u organizmu [23]. Uočena je znatna međuetnička razlika u učestalosti pojedinih polimorfizama u genu ABC1. U populaciji belaca najznačajniji je polimorfizam C3435T na egzonu 26 povezan s polimorfizmom G2677T/A na egzonu 21. Ovaj polimorfizam rezultira znatno izmenjenom funkcijom Pgp-a [35]. Polimorfizmi u genu MDR1, ne samo da utiču na ekspresiju Pgp-a, već i na koncentracije antiretrovirusnih lekova u plazmi, a samim tim na njihovu terapijsku efikasnost i bezbednost.

U tabeli 2 prikazani su geni MDR1 i genski aleli (PNP) odgovorni za transport, kao i njihov uticaj na farmakokinetiku, odnosno farmakodinamiku antiretrovirusnih lekova.

Tabela 2

Geni MDR1, pojedinačni nukleotidni polimorfizmi (PNP) odgovorni za transport i njihov uticaj na farmakokinetiku, odnosno farmakodinamiku antiretrovirusnih lekova

Gen	PNP	Lek	Farmakokinetski efekat	Farmakodinamski efekat
MDR1 (ABCB1)	2677 G>T	atazanavir	nepoznat	nepoznat
MDR1 (ABCB1)	2677 G>T	efavirenz	nepoznat	nepoznat
MDR1 (ABCB1)	2677 G>T	indinavir	nepoznat	nepoznat
MDR1 (ABCB1)	2677 G>T	lopinavir/r	nepoznat	nepoznat
MDR1 (ABCB1)	2677 G>T	nelfinavir	nepoznat	nepoznat
MDR1 (ABCB1)	2677 G>T	ritonavir	nepoznat	nepoznat
MDR1 (ABCB1)	2677 G>T	sakvinavir	nepoznat	nepoznat
MDR1 (ABCB1)	3435 C>T	atazanavir	nepoznat	nepoznat
MDR1 (ABCB1)	3435 C>T	efavirenz	kontraverzni rezulatati	nepoznat
MDR1 (ABCB1)	3435 C>T	indinavir	bez uticaja na farmakokinetiku	nepoznat
MDR1 (ABCB1)	3435 C>T	lopinavir/r	bez uticaja na farmakokinetiku	nepoznat
MDR1 (ABCB1)	3435 C>T	nelfinavir	uticaj na intracelularnu koncentraciju nelfinavira; snižene vrednosti nelfinavira u plazmi kod osoba sa CT genotipom	nepoznat
MDR1 (ABCB1)	3435 C>T	ritonavir	bez uticaja na famakokinetiku	nepoznat
MDR1 (ABCB1)	3435 C>T	sakvinavir	bez uticaja na famakokinetiku	nepoznat

Polimorfizam gena ABCC1 koji kodira sintezu transportnog proteina odgovornog za razvoj rezistencije na veliki broj lekova

Multidrug resistance associated protein-1 (MRP-1) je protein od 190 KDa, čiju sintezu kodira gen ABCC1 lociran na hromozomu 16. Farmakogenetičkim ispitivanjima nedavno je utvrđen PNP na poziciji 260 na egzonu 13 u genu ABCC1. Na poziciji 260 na genu ABCC1 je gvanin (G) u normalnom (tj. nemutiranom) obliku gena, dok je citozin (C) na istoj poziciji u mutiranom obliku gena [23].

Fiziološka uloga MRP-1 je u detoksikaciji i zaštiti organizma od oksidativnog stresa. Takođe, MRP-1 utiče na eliminaciju organskih anjona, endogenih konjugata i ksenobiotika sa glutationom, glukuronidima i sulfatima. Pored navedenih, supstrati za efluksnu pumpu MRP-1 su i antiretrovirusni lekovi iz grupe NNIRT i PI. Dalje, MRP-1 pojačava efluks ovih lekova iz ćelije, čime redukuje stepen njihove distribucije uz istovremeni porast njihove eliminacije. Na ovaj način, dolazi da sniženja njihove terapijske efikasnosti [32].

Uticaj genetičke varijabilnosti na terapijsku efikasnost antiretrovirusnih lekova izuzetno je složen. U cilju postizanja što bolje terapijske efikasnosti antiretrovirusnih lekova, kao i u cilju poboljšanja kvaliteta života i dužine preživljavanja bolesnika sa HIV infekcijom, neophodno je dodatno ispitati i utvrditi uticaj genetičkih polimorfizama na farmakokinetiku antiretrovirusnih lekova, njihovu terapijsku efikasnost i pojavu neželjenih i toksičnih efekata [33, 34, 36].

Dosadašnja ispitivanja genetičkih polimorfizama i njihovog uticaja na farmakokinetiku i terapijsku efikasnost antiretrovirusnih lekova i dalje su malobrojna. Stoga, razvoj i unapređenje znanja koje se odnosi na vezu između PNP i virusološkog, odnosno imunološkog odgovora na primenjenu kombinovanu HAART od velikog je značaja za individualni pristup u lečenju infekcije HIV. Jedan od pravaca u individualizaciji terapije mogao bi biti baziran na procesu genotipizacije gena odgovornih za metabolizam i distribuciju antiretrovirusnih lekova [37].

Zaključak

Pojedinačni nukleotidni polimorfizmi i njihove eventualne posledice na farmakokinetiku i terapijsku efikasnost antiretrovirusnih lekova još uvek su *terra incognita*, pa njihovo dalje proučavanje predstavlja značajno polje budućih naučnih istraživanja.

Gotovo svi antiretrovirusni lekovi se metabolišu pod dejstvom enzima kodiranih od strane istoimenih polimorfnih gena (citohrom P450, CYP450; glukuronil transferaza, GT) i/ili se transportuju pod uticajem proteina, tzv. transportera ABC ili SLC familije. Pokazano je da prisustvo pojedinačnih nukleotidnih polimorfizama, odnosno da pojedine varijante mutiranih gena koji kodiraju sintezu enzima odgovornih za transport (ABCB1 i ABCC1) i metabolizam (CYP 3A4, 3A5, 2B6) antiretrovirusnih lekova mogu da dovedu do pojave visokih koncentracija antiretrovirusnih lekova u plazmi i posledične pojave neželjenih i toksičnih efekata.

Zahvalnost

Izradu rada delom je podržao projekat „Filogenetski pristup analizi molekularne evolucije visokovarijabilnih virusa: koinfekcije, interakcija virusa i domaćina“, broj projekta 175024.

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Primljen 31. XII 2010.
Revidiran 17. III 2011.
Prihvaćen 23. V 2011.

CASE REPORTS

UDC: 616-006::618.1-006-06/-08
DOI: 10.2298/VSP101231031N

Developing retroperitoneal anaplastic carcinoma with choriocarcinoma focus after ovarian non-gestational choriocarcinoma: A case report

Razvoj retroperitonealnog anaplastičnog karcinoma sa horiokarcinomskim metastazama posle negestacijskog horiokarcinoma

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Abstract

Introduction. Choriocarcinoma is a malignant form of gestational trophoblastic neoplasm (GTN). It is a rare event but also a curable malignancy. In the majority of instancies it developes after any gestational event. In some cases it developes as non-gestational extrauterine malignancy. Prognosis of choriocarcinoma is poor when invasion and metastases appear early and spread fast. This form of choriocarcinoma can lead to incurable and letal outcome. **Case report.** We presented a 20-year-old patient with abdominal and retroperitoneal malignancy – anaplastic carcinoma combined with choriocarcinoma metastases in. Tumor developed three months after left adnexectomy which had been done because of adnexal tumor. Choriocarcinoma was immunohistochemicaly confirmed in adnexal masses. Two courses of chemotherapy, metotrexate + folic acid (MTX+FA) regimen, were administrated. The initial serum beta human chorionic gonadotropin level stayed unknown as well as the last one after the treatment. The patient came from the other country and was hospitalized because of pelvic and abdominal pain and palpable abdominal masses in hypogastrium with progressive anemia. The human chorionic gonadotropin level was 38 mIU/L. Tumor biopsy was done and choriocarcinoma metastases were immunohistochemicaly confirmed with predominant anaplastic carcinoma. Five day course of MTX + cyclophosphamide regimen was administrated and the patient was prepared for operative treatment. Relaparotomy was perforemed and tumor completely exceeded. Tumor mass mostly developed retroperitonely and partialy in abdominal cavity infiltrating intestinal wall with rupture of sigmoid colon. Anaplastic carcinoma, with large fields of necrosis and bleeding, was confirmed after histological examination. Immunohistochemical examination excluded choriocarcinoma in tumor mass. After 20 blood units transfusion, one course of chemotherapy and tumor excision, the patient left hospital on the 9th postoperative day. The patient rejected chemotherapy which was recommended according to the protocol and died one month after the operation. **Conclusion.** Non-gestational metastatic choriocarcinoma complicated with another type of malignancy with early spread of the disease and low responsiriness to chemotherapy has poor prognosis and leads to lethal outocome.

Key words:
choriocarcinoma; choriocarcinoma, non-gestational; carcinoma; diagnosis; drug therapy; digestive system surgical procedures; gynecologic surgical procedures; prognosis; treatment outcome.

Apstrakt

Uvod. Horiokarcinom je maligni oblik gestacijske trofoblastne neoplazme (GTN). Javlja se retko, ali spada u izlečive malignitete. U većini slučajeva razvija se nakon trudnoće. U nekim slučajevima može se razviti i kao negestacijski vanmaterični malignitet. Prognoza je loša kada se invazija i metastaze horiokarcinoma pojave rano i ako se brzo šire. Taj oblik horiokarcinoma može biti neizlečiv i dovesti do letalnog ishoda. **Prikaz bolesnika.** Prikazali smo bolesnicu, staru 20 godina, sa abdominalnim i retroperitonealnim malignitetom – anaplastičnim karcinomom sa horiokarcinomskim metastazama. Tumor se razvio tri meseca nakon leve adneksektomije koja je urađena zbog adneksalnog tumora. Horiokarcinom je imunohistohemijski potvrđen iz adneksalne mase. Date su dve kure hemoterapije metotreksatom i folnom kiselinom. Inicijalna vrednost beta humanog horionskog gonadotropina ostala je nepoznata, kao

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i poslednja nakon tretmana. Bolesnica je došala iz druge zemlje i hospitalizovana je zbog bolova u predelu abdomena i male karlice i palpabilne mase u hipogastrijumu, praćene progresivnom anemijom. Nivo humanog horionskog gonadotropina bio je 38 mIU/L. Načinjena je biopsija tumora i metastaze horiokarcinoma su imunohistohemijski potvrđene sa predominacijom anaplastičnog karcinoma. Data je petodnevna kura metotreksata i ciklofosfamida i bolesnica je pripremana za operativno lečenje. Načinjena je relaparotomija i tumor je u potpunosti uklonjen. Tumorska masa se najviše razvijala retroperitonealno, a delimično i u abdominalnoj duplji infiltrišući zid creva sa rupturom sigmoidnog kolona. Anaplastični karcinom sa velikim poljima nekroze i krvarenja potvrđen je nakon histološkog pregleda. Imunohistohemijskim ispitivanjem isključeno je horiokarcinom u tumorskoj masi. Nakon 20 jedinica transfuzije krvi, jedne kure hemioterapije i uklanjanja tumora, bolesnica je napustila bolnicu 9. postoperativnog dana. Odbila je hemoterapiju koja je bila preporučena prema protokolu i preminula je mesec dana nakon operacije. **Zaključak.** Negastacijski metastatski horiokarcinom komplikovan drugim tipom maligniteta sa ranim širenjem bolesti i slabim odgovorom na hematerapiju ima lošu prognozu sa letalnim ishodom.

Ključne reči:
horiokarcinom; horiokarcinom, negestacijski; karcinom; dijagnoza; lečenje lekovima; hirurgija, digestivni sistem, procedure; hirurgija, ginekološka, procedure; prognoza; lečenje, ishod.

Introduction

Gestational trophoblastic neoplasms (GTNs) can appear as benign GTNs (complete or partial hydatiform mole) as well as invasive mole, choriocarcinoma or placental site trophoblastic tumor as malignant GTNs. Among GTNs, choriocarcinoma is a highly potent malignancy of trophoblastic origin and usualy represents disturbance of fertilization. Choriocarcinoma is a rare event, highly malignant tumor and in the majority of instancies its localization is intrauterine and of gestational origine. Choriocarcinoma usually occurs after normal pregnancies, after term pregnancies and after molar pregnancies [1]. It can also occur after nongestational events [2, 3]. Serum beta human chorionic gonadotropin (HCG) elevation depends on hormone secretion component of choriocarcinoma (syncyciotrophoblast) [4, 5]. Extrauterine localization of choriocarcinoma is rare and can develop on ectopic pregnancy. It is believed that some malignant non-gestational trophoblastic malignancies especially choriocarcinoma can develop from pluripotent germ cells in the gonads. If non-gestational choriocarcinoma invades and metastatises early and rapidly without specific clinical manifestation it may have a poor prognosis. Diagnosis can be confirmed after histological and immunohistochemical examinations. This means that poorly differentiated carcinomas may show focal area of choriocarcinomatous differentiation [6, 7].

Case report

A 20-year-old patient presented with abdominal and retroperitoneal malignancy – anaplastic carcinoma (gravida 1, parity 1). The tumor developed three months after left adnexectomy because of cystic tumor 8 × 7 × 9 cm. CA 125 levels were 16 U/mL (< 35 U/mL ref. value). Immunohistochemical examination was done and tumor cells were identified as CK7, CK20 and beta HCG positive and diagnosis of choriocarcinoma was confirmed. The patient was treated in another country. Two courses of chemotherapy, methotrexate 50 mg/m^2 *im* on the days 1, 3, 5, 7 + folic acid 30 mg *iv* on the days 2, 4, 6, 8 (MTX + FA), were administrated without checking initial HCG level.

The patient came to our hospital with a history of pelvic pain and developing abdominal and left retroperitoneal tumor 3 months after left adnexectomy and 2 courses of chemotherapy. Right adnexectomy was done one year before this operation and histopathological findings confirmed borderline *cystadenoma ovarii*.

On admission to hospital the patient had palpable abdominal masses, pelvic and abdominal pain and progressive anemia. The tumor that developed 3 months after left adnexectomy and 2 courses of chemotherapy because of the confirmed non-gestational choriocarcinoma spreaded retroperitonealy and also in the abdominal cavity. We checked HCG and it was 38 mlU/L. Tumor marker CA-125 and alpha-fetoprotein (AFP) levels were in normal ranges. Transvaginal Doppler ultrasonographic examination was done and normal uterus without pelvic masses was seen. Hyperechogenic masses were suspected on the left lateral and back retroperitoneal and invaded to the left subphrenium (Figure 1).

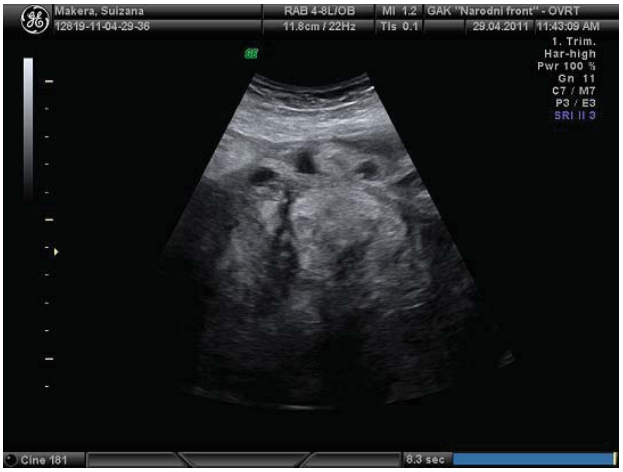

Fig. 1 – Ultrasonography of abdominal and retroperitoneal tumor mass

This was confirmed on computed tomography (CT) scans of abdomen and pelvis. CT scans of the pelvis showed the normal uterus without pathological masses and there was a heterogenous mass dimenssions 25 × 39 × 37 cm partly in ab-

dominal cavity and mostly invaded left retroperitonealy (Figure 2). Brain and lung metastases were excluded after X-ray examination.

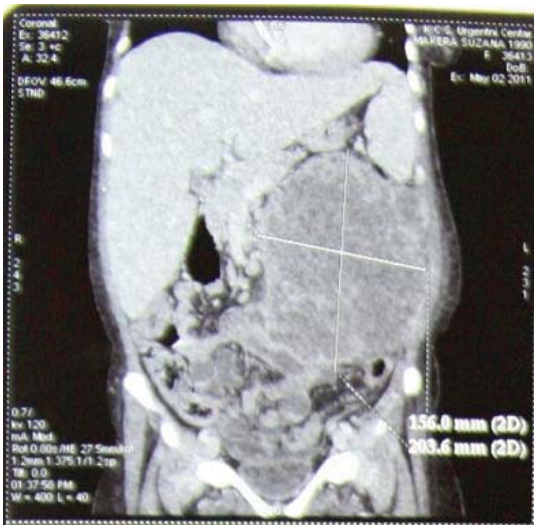

Fig. 2 – Computed tomography (CT) scan of an abdominal and retroperitoneal tumor mass

The patient had progressive anemia with hematocrite (hct) 29%–19% (reference values 37.0%–51.0%) and hypoalbuminemia 25–27 g/L (reference values 32–50 g/L). During the first 4 days the patient got 8 blood units and 12 units of fresh frosen plasma (FFP). Broad spectrum antibiotics administrated on the second day of hospitalisation when the patient became febrile (38–39.5°C) with increasing leukocytes (> 12 × 10^9/L) , platelets (> 400 × 10^9/L) and also increasing CRP > 165 mg/L (reference value < 5.0 mg/L).

To prevent multiorganic failure, endotoxic shock or/and disseminated intravascular coagulation caused by hemorrhage and infection in tumor mass we decided to exceede the tumor mass. Because of the great risk of hemorrhage biopsy of metastasis is not recommended in patients with metastatic choriocarcinoma. We decided to do it inspite of the risks, so we could make the right decision for the final treatment and to find the source of hemorrhage and suspected developing infection. Tumor biopsy was done and metastatic choriocarcinoma with predominant anaplastic carcinoma was confirmed (Figure 3). According to this and also ultrasonographic and CT findings we prepared the patient for radical tumor excision. Preparation included administrating appropriate chemotherapy regimen. Suggested chemotherapy regimens for choriocarcinoma treatment include the following: MAC regimen – metotrexate (MTX) + actinomycin–d + cyclophosphamid, then EMA/CO – etoposide + metotrexate + actinomycin-d + cyclophosphamid + vincristin. Unfortunately, these protocols could not be administrated in Serbia because actinomycin-d is not registrated.

That means that chemotherapy regimen was modified and 5 days metotrexate + cyclophosphamid regimen was administrated before total tumor excision. This therapy showed to be effective and one week later beta HCG was 2 mlU/L. Because of immunohistochemically confirmed metastatic focus of choriocarcinoma in predominant anaplastic carcinoma and only one positive HCG level of 38 mlU/mL, 5 day chemotherapy (methotrexate 15 mg IV + cyclophosphamide 300 mg IV) was administrated according to the protocol.

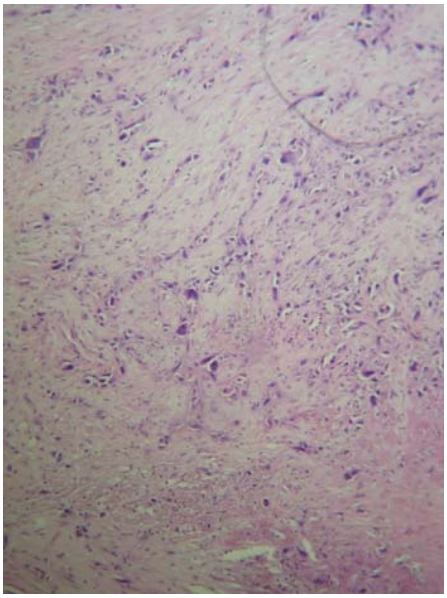

Fig. 3 – Anaplastic carcinoma (histopathologic finding of tumor biopsy post chemotherapy; HE, ×200)

One week after chemotherapy serum HCG was below 5 mlU/mL. During and after chemotherapy the patient got 8 blood units and 9 units of FFP. Broad spectrum antibiotics continued administrating. The operative treatment was done in collaboration with vascular surgeon. Rupture of sigmoid colon and hemorrhage in tumor mass were found. The tumor had been exceeded *in toto* (Figure 4). On the day before and during the operation, 12 doses of blood were transfused and 6 doses of FFP. Immunohistochemical examination excluded metastases of choriocarcinoma in the exceeded tumor mass. After chemotherapy and total tumor excision anaplastic carcinoma was confirmed with intestinal infiltration and large fields of necrosis and bleeding. Tumor cells were CK7,

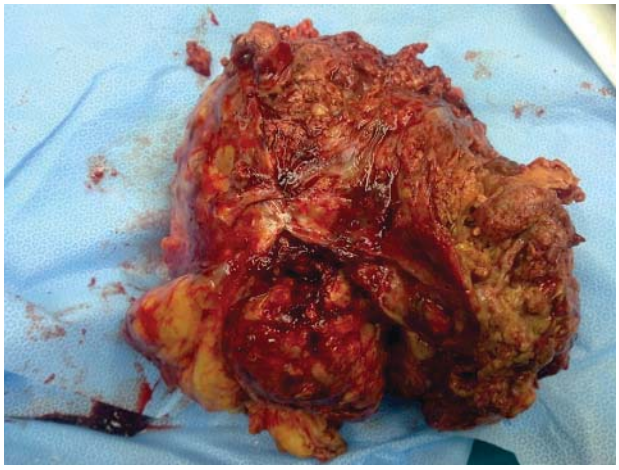

Fig. 4 – Macroscopic view of an exceeded tumor mass

CK20, beta HCG, PLAP, inhibin and p63 negative. Our patient had nongestational choriocarcinoma with poor prognosis. The patient had early and extensive development of anaplastic carcinoma with choriocarcinoma foci complicated with ruptured sigmoid colon, infection and hemorrhage in tumor tissue. This explains progressive anemia, infection and febrile state. Nine days after the operation the patient went home recovered but died one month later becouse had rejected chemotherapy recommended according to the protocol.

Discussion

Non-gestational choriocarcinoma is a rare trophoblastic malignancy. If diagnosed on time and treated it can also be curable. If it does not invade nor metastatize early the prognosis can be better and the treatment more successful. Sometimes it spreads in the abdomen and also retroperitonealy. Non-gestational choriocarcinoma are followed with significantly lower serum beta HCG than in postgestatinal choriocarcinoma. That is the reason for poor prognosis in the time of diagnosis with following complications and letal outcome [8, 9]. Because of the great risk of hemorrhage biopsy of metastases is not recommended in patients with metastatic choriocarcinoma. Destruction of local tissue and organs can be followed with progressive anemia caused by local hemorrhage in tumor, abdomen or retroperitoneum. Concommitent infection with high temperature needs antibiotic treatment and supportive therapy [10, 11, 12].

Chest and brain X-ray have to be done to exclude metastases in non-gestational as well as in gestational choriocarcinoma. Ultrasonography and CT are also of a great diagnostic value [13]. Suggested chemotherapy regimens for choriocarcinoma treatment include following: MAC regimen – metotrexate (MTX) + actinomycin–d + cyclophosphamid, then EMA/CO – etoposide + metotrexate + actinomycin-d + cyclophosphamid + vincristin. Unfortunately, these protocols could not be administrated in Serbia because actinomycin-d is not registrated.

In the presented patient the effective chemotherapy regimen was a modified one: 5 days metotrexate 15 mg *iv* + cyclophosphamid 300 mg *iv* were administrated before the operation and tumor excission. This therapy showed to be effective for choriocarcinoma foci in anaplastic carcinoma and one week later beta human chorionic gonadotropin was 2 mlU/L. Prognosis of non-gestational metastatic choriocarcinoma depends on the diagnosis as well as on treatment response. Delayed diagnosis and low responsiveness to chemotherapy and early extensive spread of disease mean poor prognosis and lead to letal outcome.

Conclusion

Non-gestational metastatic choriocarcinoma complicated with another type of malignancy with early spread of the disease and low responsiveness to chemotherapy has poor prognosis and leads to lethal outcome.

Acknowledgment

This work is a part of two projects 41021 and 175082 Ministry of Education, Science and Technological Development of the Republic of Serbia.

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Received on December 31, 2010.
Accepted on May 22, 2011.
OnLine-first, August, 2012.

CASE REPORT

UDC: 616.716.4-003.94-07-089-036
DOI: 10.2298/VSP1212101M

Peripheral ostectomy with the use of Carnoy's solution as a rational surgical approach to odontogenic keratocyst: A case report with a 5-year follow-up

Periferna osteotomija sa Karnojevim rastvorom kao racionalan pristup lečenju odontogene keratociste: prikaz bolesnika sa 5-godišnjim praćenjem

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Abstract

Introduction. Odontogenic keratocyst (OKC) is a rare developmental, epithelial and benign cyst of the jaws of odontogenic origin with high recurrence rates. The third molar region, especially the angle of the mandible and the ascending ramus are involved far more frequently than the maxilla. The choice of treatment approach was based on the size of the cyst, recurrence status, and radiographic evidence of cortical perforation. Different surgical treatment options like marsupialization, decompression, enucleation, enucleation with Carnoy's solution, peripheral ostectomy with or without Carnoy's solution, and jaw resection have been discussed in the literature with variable rates of recurrence. **Case report**. We presented a 52-year-old male with orthokeratinized odontogenic keratocyst. Elliptical unilocular radiolucency located in the third molar region and the ascending ramus of the mandible, 40 × 25 mm in diameter with radiographic evidence of cortical perforation at the anterior ramus border of the mandible 20 mm in diameter, was registrated on orthopantomographic radiography. Surgical treatment included enucleation of the cyst and peripheral ostectomy with the use of Carnoy's solution and excision of the overlying attached mucosa. Postoperatively, no paresthesia in the inervation area of the inferior alveolaris nerve was registrated. Recurrences were not registrated within 5 years post-intervention. **Conclusion.** Treatment of odontogenic keratocyst with enucleation and peripheral ostectomy with the use of Carnoy's solution and excision of the overlying attached mucosa had a very low rate of recurrence. Radical and more aggressive surgical treatments as jaw resection should be reserved for multiple recurrent cysts and when OKC is associated with nevoid basal cell carcinoma syndrome (NBCCS). Following the treatment protocol in the management of OKC and systematic and long-term post-surgical follow-up are considered key elements for successful results.

Key words:
odontogenic cysts; mandible; oral surgical procedures; recurrence.

Apstrakt

Uvod. Odontogena keratocista (OKC) je retka razvojna, epitelna, benigna cista viličnih kostiju, odontogenog porekla sa visokom stopom recidiva. Predeo donjeg trećeg molara, naročito ugla i ramusa donje vilice, zahvaćeni su znatno češće nego gornja vilica. Izbor lečenja zasnovan je na veličini cistične promene, stopi recidiva i postojanju kortikalne perforacije evidentirane radiografski. Različiti hirurški tretmani, kao marsupijalizacija, dekompresija, enukleacija, enukleacija sa Karnojevim rastvorom, periferna osteotomija sa ili bez Karnojevog rastvora i resekcija vilice razmatrani su u literaturi sa varijabilnim podacima o stopi pojave recidiva. **Prikaz bolesnika.** U radu je prikazan 52-godišnji muškarac sa ortokeratinizirajućom odontogenom keratocistom. Na ortopantomografskoj radiografiji uočavalo se elipsasto unilokularno rasvetljenje veličine 40 mm × 25 mm u predelu donjeg trećeg molara i susednog dela ramusa donje vilice sa radiografski evidentnim postojanjem kortikalne perforacije prednje ivice ramusa mandibule u rasponu od 20 mm. Operativni zahvat uključivao je enukleaciju cistične promene, perifernu osteotomiju uz upotrebu Karnojevog rastvora i eksciziju okolno zahvaćene sluzokože. Postoperativno, nije registrovan ispad senzibiliteta u inervacionoj zoni *nervus-a alveolaris inferior*. U 5-godišnjem postoperativnom periodu nije registrovana pojava recidava. **Zaključak.** Le-

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čenje odontogenih keratocista enukleacijom i perifernom osteotomijom uz upotrebu Karnojevog rastvora i eksciziju okolno zahvaćene mukoze pokazuje vrlo nisku stopu pojave recidiva. Najradikalnije i najagresivnije hirurško lečenje, kao resekcija vilice, moglo bi biti rezervisano za slučajeve čestih recidiva cista i kada je OKC udružena sa Gorlin-Golcovim sindromom. Pridržavanje terapijskog protokola u lečenju OKC i sistematično i dugotrajno postoperativno praćenje ključni su elementi uspešnog rezultata.

Ključne reči:
odontogene ciste; mandibula; hirurgija, oralna; recidiv.

Introduction

Odontogenic keratocyst (OKC) was defined as a developmental, epithelial and benign cyst of the jaws of odontogenic origin [1]. In the World Health Organization (WHO) classification of head and neck tumors from 2005, odontogenic keratocyst was reclassified and renamed to keratocystic odontogenic tumor (KCOT) [2]. Thus, this tumor was classified as a benign cystic neoplasm of the jaws of odontogenic origin [2]. The histologic features are characterized by the presence of a thin bandlike parakeratinized or (KCOT) orthokeratinized (OKC) stratified squamous epithelium [3, 4]. Although KCOT with parakeratinized epithelium have aggressive behavior with potential for rapid growth, tendency for local intraosseal destruction and penetration to adjacent soft tissue, orthokeratinized odontogenic keratocyst has different characteristics and does not show aggressive behavior [5].

The third molar region, especially the angle of the mandible and the ascending ramus are involved far more frequently than the maxilla [6].

Surgically, due to extremely vulnerable epithelium, with limited surgical access, especially in the posterior part of the mandible and ascending ramus, the existence of cortical perforation, and the desire of the surgeon to protect and preserve the integrity of vital anatomical structures, it is very difficult to remove. Because of that, both keratocystic lesions have a high rate of recurrence, which contributes to special clinical approach [7, 8].

Treatment of these lesions, even today, has a number of dilemmas and controversial opinions about the choice and the degree of radicalism in surgical procedure that is necessary to be applied, with the aim of eliminating the potential for recurrence, and minimizing the surgical morbidity [7–9]. In other words, the current dilemmas and controversies are leading to the crucial question: When and whether aggressive and radical therapy is necessary in the treatments of this cyst/tumor [9, 10]?

The aim of this report was to present a patient with large orthokeratinized odontogenic keratocystic removed by enucleation and peripheral ostectomy with additional use of Carnoy's solution and excision of the affected overlying mucosa, which was followed-up postoperatively for 5 years.

Case report

A 52 year-old male patient was admitted to the Department of Oral Surgery, Military Medical Academy (MMA) because of pain after tooth extraction which was performed elsewhere a few days ago. On the orthopantomographic radiography, an elliptical unilocular radiolucency, located in the left third molar region and the ascending ramus of the mandible, was registered. The lesion was 40 × 25 mm in diametar with radiographic evidence of cortical perforation at the anterior border of the mandibular ramus, 20 mm in diametar (Figure 1). The patient had no other clinical symptoms except pain. After the analysis of the radiographs, the patient was advised for surgical treatment after biopsy, because it is the adopted protocol for cases of the suspected KCOT[11]. The patient accepted the proposed treatment plan.

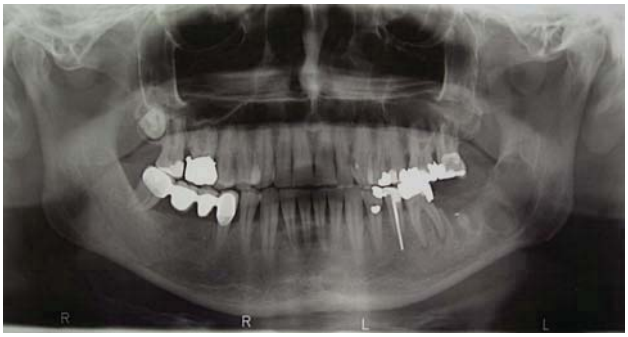

Fig. 1 – Orthopantomographic radiography before the surgical intervention

Preoperative biopsy was performed under the inferior alveolar nerve block (Articain chloride™ 4% – 3M ESPE).

The histopathological finding indicated the presence of an orthokeratinized odontogenic keratocyst (OKC). Most of the epithelium was orthokeratotic, with a mass of laminar keratin material in the lumen. The lower part of squamous epithelium was hyperplastic, due to focality inflammation (Figure 2).

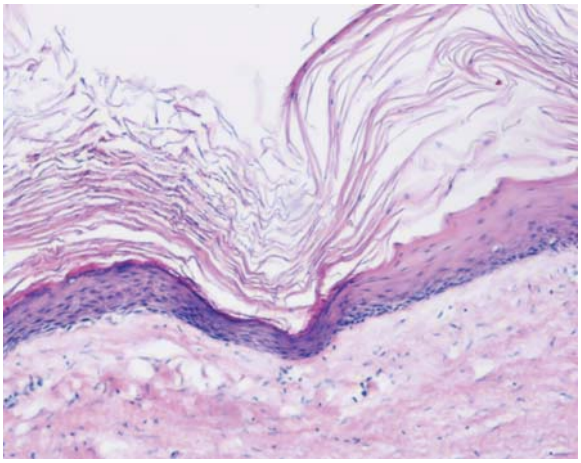

Fig. 2 – Hyperkeratotic cyst's wall (H&E, × 75)

Upon receipt of the histopathological findings, the patient was advised for hospitalization and surgery to remove

the existing lesion under general anesthesia. In the Clinic for Maxillofacial, Oral Surgery and Implantology, MMA, all the necessary laboratory analysis for surgery under general anesthesia were made, and at the Institute of Pharmacy, MMA, Carnoy solution was prepared according to the following prescription: absolute alcohol (6 mL), chloroform (3 mL), glacial acetic acid (1 mL) i ferric chloride (1 g).

Surgical procedure was started with incision along the anterior border of the left mandibular ramus, then over the alveolar crest to the gingival margin till the tooth 35, with vertical relaxing incision down to the fornix in this region. After uplifting the mucoperiostal flap and separation of the masseter muscle and the pterygoid medial muscle attachments defect of the anterior border of ramus about 20 mm in diametar was seen (Figure 3). The defect was slightly wid-

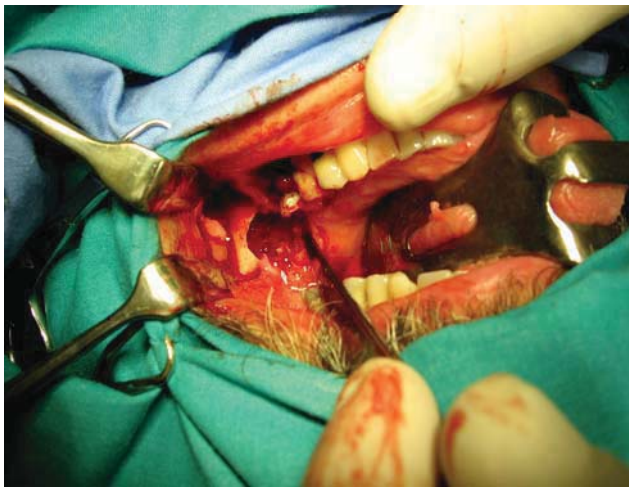

Fig. 3 – Intraoperative features: cortical perforation at the anterior ramus border of the mandible

ened caudally at the lateral aspect of the mandibular ramus, in order to approach the lesion. It was completely enucleated, a defect rinsed with saline, and a sterile gauze swab presoaked with Carnoy's solution was placed in the lumen of the defect and left there for 3 minutes. Then, the lumen of the defect was re-rinsed with saline to enable sight of the cystic wall remains, which were dark brown colored and fixated, enabling their complete removal. After that, a peripheral ostectomy in the caudal and cranial direction was performed and the overlying attached mucosa was excised. After repeated rinsing with saline, the defect was buffered with the iodine-vaseline gauze and the wound was suturated.

Postoperatively, the patient was prescribed intravenous antibiotics: ceftriaxon 2 g once daily and metronidazol 0.5 g three times daily, for the folowing seven days. The patient was regularly controlled, every day. The whole iodine gauze packing was removed at the third postoperative day and the sutures were removed after 7 days. No paresthesia in the inervation area of the inferior alveolaris nerve was present, as well as any other postoperative complications. The patient was followed up regularly, and after a 5-year period, orthopantomographic radiography was done again (Figures 4 and 5).

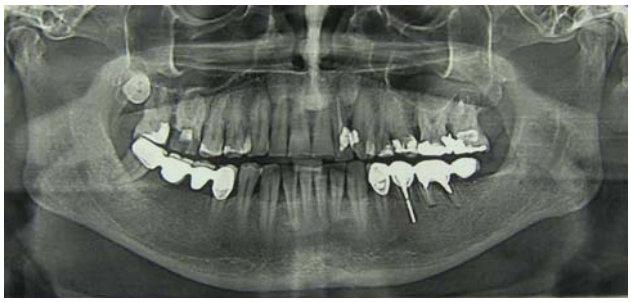

Fig. 4 – Orthopantomographic radiography 5 years after the surgical intervention

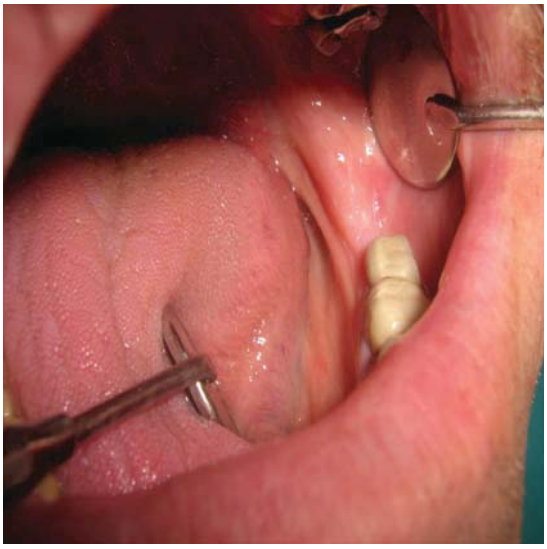

Fig. 5 – Intraoral view 5 years after the surgical intervention

Discussion

OKCs are primary jaw bone lesions, but it is interesting to note that these cysts can occur in highly atypical places [12]. Eryilmaz et al. [13], showed the occurrence of OKC in the temporomandibular joint, while Yih and Krump [14] registered such cyst in nasopalatinal duct as a very rare occurrence. Also, some authors reported a case of peripheral OKC, outside the jaws, in the gingival tissue [15, 16] or even in the buccal mucosa [17].

OKC treatment involves surgical approach only. Generally, treatments are classified as conservative or radical (aggressive). Conservative methods include: simple enucleation (with or without curettage), marsupialization with subsequent enucleation, and decompression with subsequent enucleation. Aggressive methods include: cryotherapy, the use of Carnoy's solution, peripheral ostectomy and resection of the jaw with or without immediate reconstruction of a defect with corticocancellous iliac crest bone allografts [7, 18–20].

The most significant and important therapeutic problem, related to these cysts is the high rate of recurrence after initial surgical treatment. Boyne et al. [21] and Chemli et al. [22] reported the frequency of recurrences up to 60%, while some authors have registered recurrence after 25 years [23], or even 41 years after the first surgical intervention [4]. The most

common reason for recurrence is incomplete removal of cyst walls or epithelial islands and/or microcysts, in the peripheral bone and the overlying soft tissue [23]. Stoelinga [23] stated that in approximately 50% of islets of a cyst wall and epithelium and/or microcysts remain in the overlying soft tissue, particularly in cases of cortical perforation.

Literature data suggest that recurrences appear most frequently within the first 5 to 7 years after initial surgery [8, 18, 19, 21–23]. Chirapathomsakul et al. [24] reported the frequency of recurrence by as much as 71.4% in the first five years. Also, Pitak-Arnnop et al. [9] reported that the thirty-two of the 37 recurrences occurred within 5 years. However, as stated by Crowley et al. [4], more than 25% of recurrences occur after 9 years or even later after the first surgery.

It seams that age of the patient, localization of OKC and site of involvement, its histological type (orthokeratosis or parakeratosis), do not significantly affect the incidence of recurrence [9, 19, 24, 25]. In other words, the recurrence rate mainly depends on the applied surgical methods. However, the choice of surgical treatment depends on several factors, such as the size of the lesion, frequency of recurrence and radiographic evidence of cortical perforation [6, 7, 19]. Pitak-Arnnop et al. [9] reported that the recurrence was found in 28 patients and 11 of these had cortical perforation at the time of first presentation. Because of that, a crucial point in the treatment of OKCs is an adequate or appropriate choice of surgical method [7, 8, 19, 20].

However, the treatment of OKC remains controversial. There are different data on the incidence rate of recurrence after different surgical methods of treatment. There are several explantations of such diversity. First of all, OKC is extremly rare, so the number of samples used in study is usually little. Furthermore, most studies have retrospective nature, often lacking valid and adequate clinical data and control groups. Finally, a period of follow-up of operated patients is highly variable in different studies [7, 8, 19, 20].

Although it is stated that patient age does not influence the recurrence, it can be a very important factor in the choice of surgical treatment. The choice of conservative methods, despite high rates of recurrence, may be justified if there is a risk of injury to the surrounding anatomic structures [26–30], especially in young patients, because the use of aggressive surgical techniques in children can cause disturbances in the growth and development of jaws and teeth [27].

However, although some authors report positive clinical experience using conservative methods (marsupialisation, decompression methods and simple enucleation) in the surgical treatment of these cysts [9, 20, 28–31], we believe that it cannot be the definitive surgical method to treat OKC due to extremely high rates of recurrence, which is the attitude of many authors [7, 8, 10, 19, 23]. Also, some authors suggest that enucleation, without Carnoy's solution, provides clinically acceptable and satisfactory results with a recurrence rate of 13.3% [24] or 26% [9]. Tolstunov and Treasure [20] believe that the histological diagnosis of OKC does not indicate immediate rehearsal of surgery because only 25%–50% of the cases recur after simple enucleation, and suggest that a "wait and see" protocol should be applied. However, many authors suggest that simple enucleation as surgical option without Carnoy's solution is not adequate because of high recurrence rates of 50% [32], or 54.5% 8 or to 62% [33].

Recurrence rate after surgical treatment of these lesions is certainly a big therapeutic problem. Therefore, radical surgical intervention with the use of Carnoy's solution and radical removing the soft tissue is sometimes suggested [34]. In our view, for lesions with such aggressive behavior, regardless its "benign" nature, a more aggressive and radical surgical approach is needed. However, our opinion is that resection of the jaw, as the most radical and aggressive therapeutic option, should be used in the treatment of OKCs only in cases of frequent recurrences (more than three) and in situations when OKC is associated with nevoid basal cell carcinoma syndrome. There are two major reasons for this: resection of the jaw causes significant functional and aesthetic problems to the patient and less aggressive surgical methods, such as peripheral ostectomy with the use of Carnoy's solution, also provide an extremely low rate of recurrence, without functional and aesthetic problems.

Peripheral ostectomy was defined as a peripheral bone reduction with powered hand-piece and rotary instruments, done after enucleation of the lesion [8]. This method can be combined with the use of Carnoy's solution, which acts as an cauterizing agent, causing denaturation of organic molecules. Its penetration into the tissue results in rapid local fixation and hemostatic action [35]. Some authors suggest that the major disadvantages of Carnoy's solution are its systemic toxicity and local caustic effect resulting in a damage of vital anatomical elements (especially nerves) [25]. However, Blanas et al. [18] state that application of Carnoy's solution to cyst cavity for 3 min after enucleation should not damage the inferior alveolar nerve.

Certainly, the use of Carnoy's solution as an adjunct measure in surgical treatment of OKC and its correct application is of non questionable importance [7, 32]. This is corroborated by the studies in which comparisons were performed with the recurrence rate of application of certain surgical method with and without Carnoy's solution [7, 32].

A recent evidence reveals that most of epithelial islands and/or microcysts, as the most crucial factors for recurrence, are in the overlying mucosa that contacts a cyst, especially in the mandibular retromolar area [7]. Therefore, the essence of Carnoy's solution use lies in its influence on epithelial islands and microcystic formations; a consequent peripheral ostectomy with excision of the overlying attached mucosa completely eliminates the presence of epithelial changes [7, 19, 20, 23, 24, 34].

The results of studies that compared the incidence of recurrence of different surgical techniques in OKC showed that in cases of peripheral ostectomy combined with Carnoy's solution no recurrences were noted in ten years and more postoperatively [7, 8, 19].

Conclusion

In the presented case operated by enucleation of the lesion with peripheral ostectomy and the use of Carnoy's solution, including excision of the overlying attached mucosa, no

recurrence was noted in a 5 year postoperative period. Therefore, we believe that the choice of this surgical method in treating odontogenic keratocyst is a rational approach to reduce recurrence. However, due to the possibility of late recurrence, a long-term postoperative follow-up is needed to confirm a successful result of the OKC treatment.

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Received on April 27, 2011.
Revised on June, 14 2011.
Accepted on June 27, 2011.

CASE REPORT

UDC:618.19-006-06::616.441
DOI: 10.2298/VSP1212106K

Rare localisation of breast cancer metastasis to thyroid gland

Retka metastaza karcinoma dojke u tiroidnu žlezdu

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Abstract

Introduction. Metastases to the thyroid gland are very rare. They are usually seen in malignant melanoma, kidney, breast cancer and lung cancer. **Case report.** We presented a 54-years-old female patient with breast cancer diagnosed in 2002. The adequate surgical procedure was done and the tumor and axillary lymph nodes were removed. The patient also received adjuvant postoperative chemotherapy. After seven years of a disease free period, the first relapse of the disease was detected as thyroid gland tumor with axillary lymphadenopathy. The patient had a good response to systemic treatment so the surgical removal of thyroid gland and enlarged lymph nodes was performed. Histopathological analysis confirmed metastasis with breast cancer origin. Radical mastectomy was also preformed. Second relapse of the disease was detected 10 months later, while the patient was on hormonal therapy. It was manifested as the appearance of bone and skin metastases, pleural effusion and lymphadenopathy. **Conclusion.** This case report emphasized the importance of detailed examination of any new onset of thyroid swelling in a patient with previous history of malignancy.

Key words:
breast neoplasms; neoplasm metastasis; thyroid gland; diagnosis, differential; immunohistochemistry.

Apstrakt

Uvod. Metastaze u štitnoj žlezdi veoma su retke. Najčešće se viđaju kod malignog melanoma, karcinoma bubrega, karcinoma dojke i karcinoma pluća. **Prikaz bolesnika.** Kod bolesnice, stare 54 godine, postavljena je dijagnoza karcinoma dojke 2002. godine. Sprovedeno je adekvatno hirurško lečenje pri čemu su uklonjeni tumor i limfni čvorovi iz istostrane pazušne jame. Bolesnica je primila i adjuvantnu postoperativnu citostatsku terapiju. Nakon sedam godina došlo je do prvog relapsa bolesti koji se manifestovao pojavom čvora u štitnoj žlezdi i uvećanim limfnim čvorovima u pazušnoj jami. Bolesnica je dobro odgovorila na sistemsku hemioterapiju, pa je sprovedeno hirurško uklanjanje štitne žlezde i uvećanih limfnih čvorova. Patohistološka analiza potvrdila je da se radi o metastazi karcinoma dojke. Takođe, učinjena je i radikalna mastektomija obolele dojke. Drugi relaps bolesti registrovan je nakon 10 meseci, dok je bolesnica bila na hormonskoj terapiji. Detaljnom procenom bolesti potvrđeno je prisustvo metastaza u kostima, na koži, pleuralni izliv i uvećenje limfnih čvorova. **Zaključak.** Ovaj prikaz ukazuje na važnost detaljnog ispitivanja svakog novonastalog uvećanja štitne žlezde kod bolesnika sa prethodnom istorijom malignog oboljenja.

Ključne reči:
dojka, neoplazme; neoplazme, metastaze; tireoidna žlezda; dijagnoza, diferencijalna; imunohistohemija.

Introduction

Metastasis to the thyroid gland is usually considered rare [1, 2]. The overall incidence in autopsy series has been 0%–1% in unselected autopsy studies and around 24% in patients with metastatic disease [3–8]. The most common among metastasizing cancers to the thyroid gland are malignant melanoma, kidney, breast cancer and lung cancer [9]. We presented a female patient with local recurrence of breast cancer and metastasis in the thyroid gland, without any other distant metastases.

Case report

A 54-year-old woman was diagnosed with carcinoma of the right breast in 2002 at the Institute for Oncology and Radiology of Serbia, Belgrade, Serbia. After initial biopsy, tumorectomy with axillary dissection was performed in stage T2N1. Histopathological analysis confirmed ductal carcinoma with lobular component. Oestrogen receptors were 30% positive, progesterone 10% and HER2/neu was negative at immunohistochemistry. Malignant cells were found in one out of nine lymph nodes. The patient received six cycles of

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adjuvant chemotherapy with CMF protocol, containing cyclophosphamide, methotrexate and 5-fluorouracil. Postoperative radiotherapy was also administered.

The patient was followed up every six months for the period of seven years. No local or distal recurrence was found until the beginning of 2009, when the first relapse of the disease was detected as local recidive in the right breast with both side axillary lymphadenopathy. At that time, Estern Corporative Oncology Group/World Health Organization (ECOG/WHO) performance status of patient was 0. The treatment was continued with systemic chemotherapy according to FAC regimen (5-fluorouracil, doxorubicin and cyclophosphamide). After the first cycle of chemotherapy, thyroid gland became physically palpable so additional examination was done. Ultrasonography showed low density, 3 cm sized nodule in the left lobe of the thyroid gland, with no calcification. The thyroid hormone levels in blood were within normal ranges. The patient did not have any symptoms such as dyspnoea or wheezing present. After six cycles of chemotherapy, loboisthmectomy was performed in June 2009. Biopsy *ex tempore* showed metastatic breast cancer cells in thyroid gland tissue (Figure 1). Definite histopathological confirmed two foci of breast cancer metastasis. In addition, immunohistochemical analysis confirmed: oestrogen receptor (ER) Allred score 8, progesteron receptor (PR) score 7, HER2/neu 1+ (Figure 2), CK7+, CK19+. The tests were negative for thyroid transcription factor-1 (TTF-1) (Figure 3), tireoglobuline, vimentine and monoclonal antibody HBME-1. The struma was also seen. During surgical procedure, enlarged lymph nodes were removed and breast cancer metastases were also found in 3/5 supraclavicular and 2/3 jugular lymph nodes. The treatment was continued with six cycles of weekly paclitaxel and hormonal therapy tamoxifen. After the partial response was achieved, radical mastectomy of the right breast was performed in April 2010. Histopathology confirmed invasive lobular carcinoma grade 2, ER score 8, PR score 7, HER2/neu negative (score 1+) (Figure 4). The patient continued to be on hormonal therapy until March 2011 when the new evaluation of the disease was performed. Computed tomography body scan (PET CT) showed multiple metastases in bones. Chest radiography revealed pleural effusion. Cutaneous lenticular metastases and axillary lymphadenopathy were seen on clinical examination. The only symptom that the patient reported was pain in the lower spine. ECOG/WHO performance status was 1.

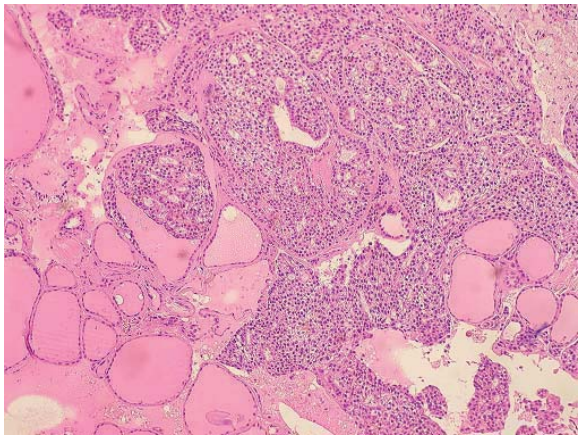

Fig. 1 – Breast cancer metastasis in the thyroid gland tissue (*Ex tempore* diagnostics; HE, ×10)

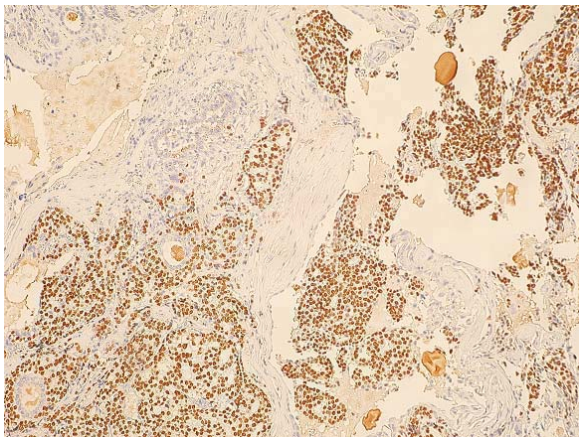

Fig. 2 – Oestrogene receptor, immunocytochemical staining (Allred score 8)

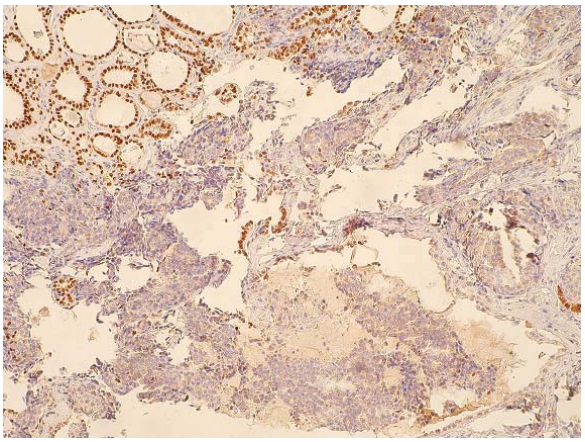

Fig. 3 – Tumor tissue negative, surrounding the thyroid gland tissue positive (immunocytochemical staining for thyroid transcription factor-1)

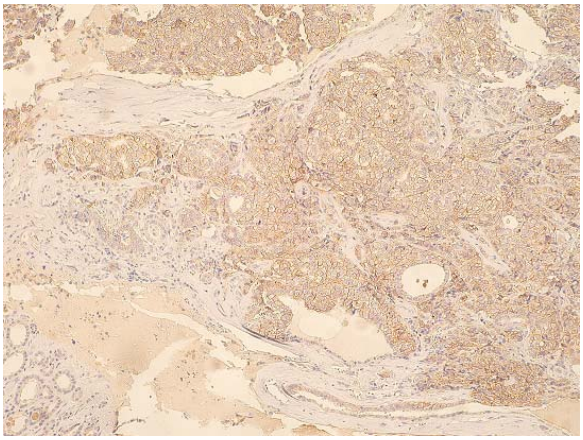

Fig. 4 – c-erbB2 receptor (HER2/neu) immunocytochemical staining (score 1+)

The patient was presented to a multidisciplinary team at the Institute for Oncology and Radiology of Serbia. It was decided to interrupt tamoxifen and continue the treatment with aromatase inhibitors. Palliative radiotherapy of the cervical spine and pelvis bones was also planned.

Discussion

The incidence of metastatic disease to the thyroid gland has been reported to be 0%–1% in unselected autopsy studies and around 24% in patients with confirmed metastatic disease [3–8]. As a result of the lack of awareness among clinicians, clinical diagnosis is even less common than *postmortem* findings.

Although the thyroid gland could be the only site of malignant disease, usually most of patients with thyroid metastases have widespread metastatic disease. Therefore, detection of metastasis to the thyroid gland often indicates poor prognosis. In a small percentage of patients, early diagnosis and aggressive surgical or medical therapy probably may be effective and contribute to the prolonged survival [10–12]. There is still no precise consensus, but a thyroid lobectomy and/or isthmectomy is recommended in case of solitary metastasis and a total thyroidectomy if thyroid metastases are bilateral [13].

A long interval between the diagnosis of primary cancer and subsequent thyroid gland metastasis can represent a diagnostic dilemma. Therefore, the standard for all newly incurred thyroid swelling in a patient with previous history of malignancy, regardless the duration of that period, should be considered as recurrence until proved otherwise [14]. In patients with metastases, low performance status and poor prognosis, fine needle aspiration biopsy (FNAB) can be used to detect an unsuspected malignancy and to avoid unnecessary thyroidectomy [7].

Conclusion

This report emphasizes the importance of detailed examination of any new onset of thyroid swelling, especially in a patient with previous history of malignant disease.

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Received on April 29, 2011.
Accepted on November 17, 2011.

CASE REPORT

UDC: 616.711-002.5
DOI: 10.2298/VSP1212109M

Subdural tuberculous abscess of the lumbar spine in a patient with chronic low back pain

Subduralni tuberkulozni apsces lumbalne kičme kod bolesnice sa hroničnim lumbalnim sindromom

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Abstract

Introduction. Despite modern imaging methods, tuberculous abscess in the subdural space of the spine can lead to misdiagnosis and to neurogical complications development, even more up to paraplegia. We presented an extremely rare case of subdural tuberculous abscess of the lumbar (L) spine and paraparesis in imunocompetent a 49-year-old patient. **Case report.** A patient with chronic L syndrome and a history of intervertebral (IV) disc L3 and L5 operations got severe back pain late in July 2007. At the same time the patient had a purulent collection in the left knee, and was treated with high doses of corticosteroids and antibiotics. Then, the patient got a high fever, the amplification of pain in the L spine and the development of paraparesis. Erythrocyte sedimentation rate was 108 mm/1 h, C-reactive protein 106.0 mg/L, white blood cell (WBC) 38.4 × 09/L with a left turn. Magnetic resonance imaging (MRI) of the spine was registered expansive formation in the spinal canal, from the level of the IV disc L2 to the mid-L4 vertebral body. This finding is a "spoke" in favor of the extrusion and sequestration of IV disc L3 with the cranial and caudal migration. The patient underwent an emergency neurosurgical operation. The diagnosis of subdural staphylococcal abscess of L spine was made. According to the antibiogram antibiotic therapy was applied but without effect on the course of the disease. Control MRI of the L spine showed spondylodiscitis L3/L4, abscess collection in the spinal canal and paravertebral muscle abscess. Late in September 2007 the patient underwent needle biopsy of the L3 vertebral body guided by computed tomography and the acid-fast bacilli (AFB) were found. Tuberculostatics were introduced in the therapy. Two years later the patient was without significant personal difficulties, and with normal clinical, laboratory and morphological findings. **Conclusion.** Subdural tuberculous abscess of the spine is extremely rare manifestation of spine tuberculosis. The exact and early diagnosis and adequate treatment of atypical form of spine tuberculosis are key factors of good prognosis.

Key words:
tuberculosis; abscess; subdural space; lumbar vertebrae; diagnosis, differential.

Apstrakt

Uvod. Subduralni tuberkulozni apsces kičme može biti nedijagnostikovan i praćen neurološkim komplikacijama sve do paraplegije, uprkos modernim radiološkim metodama. Prikazan je izuzetno redak slučaj subduralnog tuberkuloznog apscesa lumbalne (L) kičme sa paraparezom, kod imunokompetentne bolesnice, stare 49 godina. **Prikaz bolesnika.** Bolesnica sa hroničnim L-sindromom i anamnezom o operacijama intervertebralnog (IV) diska L3 i L5 dobila je jake bolove u L-kičmi krajem jula 2007. Istovremeno, imala je i gnojnu kolekciju u predelu levog kolena. Lečena je visokim dozama kortikosteroida i antibioticima. Tada je dobila visoku temperaturu, uz pojačanje intenziteta bola u L-kičmi i razvoj parapareze. Sedimentacija eritrocita bila je 108 mm/1 h, C-reaktivni protein 106,0 mg/L, leukociti 38,4 × 109/L sa skretanjem ulevo. Magnetnom rezonancom (MR) L-kičme u spinalnom kanalu, počev od IV diska L2 do polovine tela pršljena L4 registrovana je ekspanzivna formacija. Nalaz je ukazivao na ekstruziju i sekvestraciju IV diska u nivou IV prostora L3 sa kranijalnom i kaudalnom migracijom. Urađena je hitna neurohirurška operacija koja je pokazala da se radi o subduralnom stafilokoknom apscesu L-kičme. Prema antibiogramu primenjena je antibiotska terapija, ali bez efekta na tok bolesti. Kontrolna MR L-kičme pokazala je spondilodiscitis L3 i L4 pršljena, apscesnu kolekciju u spinalnom kanalu i apsces paravertebralne muskulature. Krajem septembra 2007 urađena je iglena biopsija tela L3 pršljena vođena kompjuterizovanom tomografijom i dokazani su acidorezistentni bacili. U terapiju su uključeni tuberkulostatici. Dve godine kasnije bolesnica je bila bez značajnijih subjektivnih tegoba, sa normalnim kliničkim, laboratorijskim i morfološkim nalazima. **Zaključak.** Subduralni tuberkulozni apsces kičme je ekstremno retka manifestacija tuberkuloze kičme. Tačna i rana dijagnoza i adekvatna terapija atipične forme tuberkuloze kičme ključni su faktori dobre prognoze.

Ključne reči:
tuberkuloza; apses; subduralni prostor; pršljenovi, lumbalni; dijagnoza, diferencijalna.

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Introduction

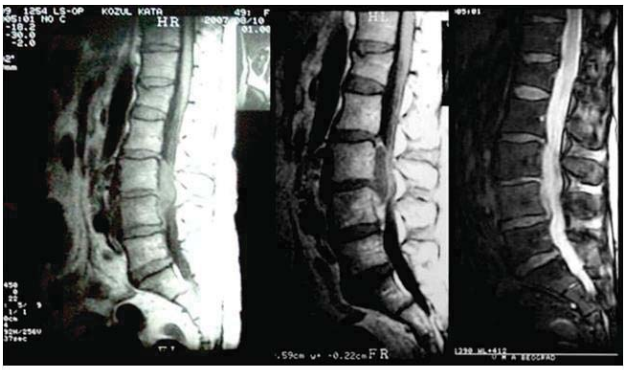
Tuberculosis (TB) of the spine accounts for about 2% of all cases of TB and for almost 50% of all patients with skeletal TB [1–3]. The disease can affect one or more parts of the spine, vertebral body, intervertebral (IV) disc, paravertebral soft tissue and/or epidural space [3–8]. Rare cases of intramedullary and extramedullary tuberculoma and tuberculous abscess with spinal cord compression, without radiological signs of tuberculous spondylitis and meningitis are also described [9–14]. However, the development of tuberculous abscess in the subdural space of the spine is extremely rare [15–18]. Despite modern imaging methods, atypical presentations of spinal TB can lead to misdiagnosis and the development of neurological complications, sometimes up to paraplegia [4, 6, 8, 19–24]. We presented an extremely rare case of subdural tuberculous abscess of the lumbar spine, and paraparesis.

Case report

In late July 2007, a 49-year-old patient with chronic lumbar (L) syndrome and a history of of IV disc L3 and L5 operations felt severe back pain. During this period, after an injury, the patient developed a purulent collection in the area of the left knee that was drained with the use of antibiotic therapy. At the same time, because of severe pain in the L spine, the patient received high-dose of methylprednisolone from August 2–4. Suddenly, strong chills and 40ºC temperature appeared in the patient. The back pain intensivied and spread out along both legs, followed by the occurrence of paresthesia in the toes of both feet and inability to walk. Therefore, the patient was admitted to the Clinic for Neurosurgery, Military Medical Academy (MMA), Belgrade on August 9. She felt tenderness on pressure and paravertebral muscle spasm in the lower part of the L spine. The clinical examination revealed high temperature, crossed sign of Lazarevic and hypoesthesia in the left dermatomes L3, L4 and L5. A rough main force of the dorsal flexors on the left foot has been weakened. A purulent collection was noticed in the area of the left knee. In laboratory studies on admission leukocytes were 38.4 × 109/L and neutrophils were 35.0 × 109/L.

Magnetic resonance imaging (MRI) of the spine, from the level of IV disc L2 to the mid-L4 vertebral body, revealed "an extradural", expansive formation with signal intensity of IV disc in the spinal chanal (Figure 1). This formation was occupying the anterior two thirds of the spinal chanal. Following administration of contrast, the formation increased signal intensity in the T1W sequence just marginally. This finding was a "spoke" in favor of the extrusion – sequestration of IV disc L3 with the cranial and caudal migration.

Soon after the admission, incision and drainage of the purulent collection were done in the region of the left knee. Unfortunately, we did not test the culture of pus, and the patient received ceftriaxone, 2,000 mg daily intravenously. This therapy resulted in regression of the purulent collection of the knee, however, the fever and increased leukocytes count maintained continuously. Despite severe back pain and



a. b. c.

Fig. 1 – Magnetic resonance imaging of the spine: T1W sequence with and without contrast (a,b); T2W FSE sequence (c) – expansive formation of the spinal canal at the level of L3/L4 vertebral bodies

neurological deterioration the patient underwent neurosurgical intervention on August 17. On that occasion, laminectomies L3 and L4 were performed and then dural sack was moved. Unexpectedly, dural tearing and leakage of purulent content from the expansive formation into the spinal canal were found. Curettage of the abscess cavity was performed immediately and the dura was sutured. *Staphylococcus aureus* sensitive to most antibiotics was isolated from the purulent content. The antibiotic therapy was continued using vancomycine in a dose of 2,000 mg a day, and the diagnosis of spinal subdural *Staphylococcus aureus* abscess was made.

In order to continue the treatment, the patient was transferred to the Clinic for Infectious Diseases, MMA, on August 23, 2007. At the admission, the patient had high fever, and was hardly moving because of severe pain in her back and legs. In laboratory studies erythrocyte sedimentation rate (ESR) was 108 mm/1.h, C-reactive protein (CRP) 106.0 mg/L, leukocytes 13.2 × 10^9/L, neutrophils 78.3%, erythrocytes 3.3 × 10^{12}/L, hemoglobin 99 g/L, platelets 616 × 10^9/L, albumin 17 g/L. Control MRI of the L spine in early September showed edema and inflammation of L3 and L4 vertebral bodies with signs of discitis and thickening of the dura mater at the level of L4 (Figure 2). Paravertebral puru-

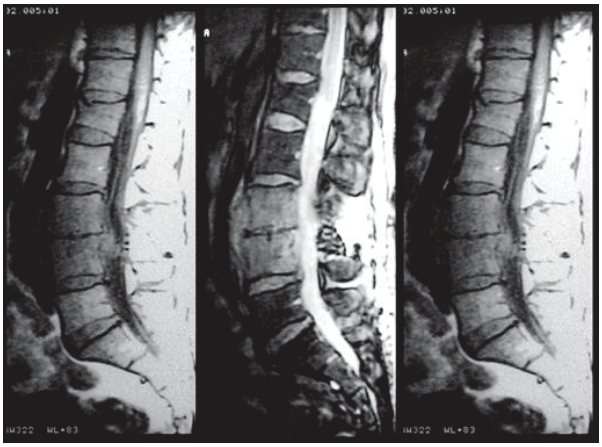


Fig. 2 – Magnetic resonance imaging of the spine (September 4, 2007) shows spondylodiscitis L3/L4, subdural and prevertebral abscess

lent collections around the vertebral bodies L3 and L4 and the abscess collection in the spinal canal, lower than the baseline were also registered.

The combined use of glycopeptides and carbapenems, followed by other antibiotics was continued (Figure 3). However, the fever, back pain and high values of ESR (100 mm/1.h) and CRP (43.5 mg/L) were still held. Due to lack of response to this therapy, the patient underwent needle biopsy of the vertebral body L3 guided by computer tomography on September 27. Using direct observation of the obtained material, acid-fast bacili (AFB) were seen, suspected on *Mycobacterium tuberculosis* (MBT). On the following day, the treatment was continued by using four first-line of tuberculostatics (streptomycin – 1,000 mg daily, isoniazid – 500 mg daily, rifampicin – 600 mg daily, ethambutol – 1,200 mg daily). This therapy leads to a gradual normalization of body temperature, thus reducing the patient's discomfort problems and normalization of laboratory findings (Figure 3). Polymerase-chain reaction (PCR) results on *Mycobacterium tuberculosis* and culture of biopted specimens by Löwenstain, as well as MBT cultures from vertebra and paravetebral abscess collections, which were subsequently obtained, were negative. Malignant cells were not registered using cytological examination of the tissue obtained by biopsy, and its histological analysis indicated that it was a blood clot.

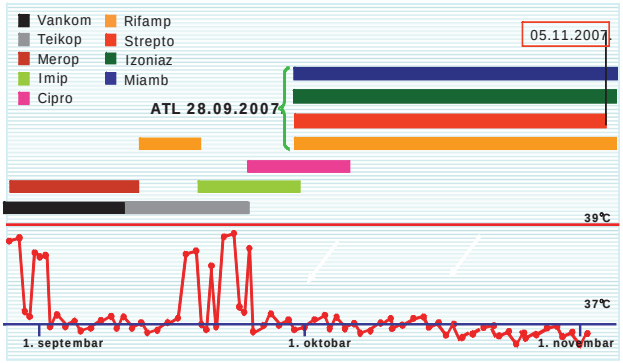


Fig. 3 – The course of the disease and treatment of patients with subdural tuberculous abscess of the lumbar spine

Control MRI of the L spine was done in late October 2007. The slight regression of spondylodiscitis L3/L4 was registered, compared to the previous review. The collapse of the IV disc and progression of destruction IV space L3 were recorded, too. Subdural and paravertebral fluid collections were not identified. Since, November 5, 2007, the patient continued the treatment by three tuberculostatics, without streptomycin for the a total of 18 months (Figure 3). This therapy was conducted daily for 12 months. After that, the same drugs were administered three times a week for three months, and then twice a week. Upon completion of the therapy, the patient was without significant subjective symptoms, and clinical findings and laboratory tests were within normal limits. There were no signs of spondylodiscitis, and subdural or paravertebral fluid collection on MRI of the L spine (Figure 4).

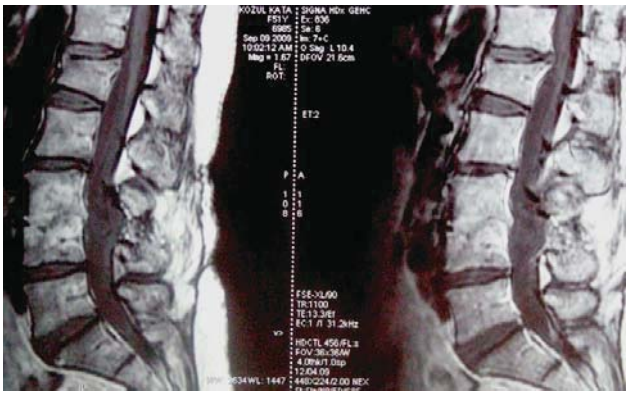


Fig. 4 – Magnetic resonance imaging of the spine (September 9, 2009) in the patient with subdural tuberculous abscess after therapy completion shows a complete regression of the disease

Discussion

In this paper, we presented an extremely rare case of subdural tuberculous abscess of the L spine, as a serious differential diagnostic problem. Generally, the classic form of spinal TB (Pott's disease) is easily recognized and treated promptly. Radiological methods revealed frontal destruction of two adjacent vertebrae with destruction of the corresponding IV disc and kyphotic deformity. Often, there is a bilateral psoas abscess, and all patients have compression of the spinal cord or cauda equina [25–28]. On the other hand, the atypical forms of spinal TB, which accounts for nearly 25% of all patients with this disease, may represent an important problem. Despite modern radiological methods and therapy, they are accompanied with frequent development of neurological complications, because of delayed diagnosis, which sometimes can lead up to paraplegia [4, 6, 8, 19–23]. Atypical forms of spine TB are a tuberculous spondylitis without involvement of IV disc, TB of posterior spinal elements or different parts of the spine and destructive lesions of cervical vertebrae and the sacrum [6–8, 23, 29, 30]. Cases of spine TB with signs of compression of the spinal cord without radiological evidence of tuberculous spondylitis or meningitis have been described very rarely [9–13, 23].

The development of spinal subdural abscess, particularly of tuberculous origin, has been recorded extremely rare. Velissaris et. al. [18] pointed out that a total of 65 cases of spinal subdural abscess had been reported in the literature until the first half of 2009. According to the same authors, *Staphylococcus aureus* was the most common bacterial cause of this disease, in 35 patients, while *Mycobacterium tuberculosis* was causative agent in only two cases [18]. However, in both of the above cases, the development of tuberculous spinal subdural abscesses occurred during the course of tuberculous meningitis, which facilitated the diagnosis [15, 16]. All these facts about atypical spine TB are sufficient to clarify why our patient was initially misdiagnosed. In addition, wrong diagnosis is ascribed to the fact that patients suffer from chronic low back pain for many years. Also, the presented patient was operated for IV disc herniation twice. The appearance of high fever and leukocytosis were initially in-

terpreted with the development of sepsis from purulent foci in the region of the left knee, after the administration of high doses of corticosteroids. Therefore, no surprise, that the spinal subdural abscess was initially misdiagnosed as IV disc extrusion, in this case.

In recent years, "medical awareness" of spine TB is less than the actual incidence of the disease. Late set of exact diagnosis is the main cause of permanent damage to the high rate and prolonged duration of illness [31–33]. Therefore, it is important to know that extensive abstraction of the spinal canal and compression of the spinal cord, in the absence of radiological signs of vertebral infection, indicate spine TB [19–25]. The authors from the MMA [34] have previously described spinal subdural abscess caused by *staphylococcus*. This may be another reason for this rare entity to be associated with possible staphylococcal infection in the area of the left knee. Unfortunately, it has been confirmed by *Staphylococcus aureus* isolation from the surgical site. At the same time it was justified to continue the patient treatment with glycopeptides. However, over time it was shown that it was most likely, a contamination. On the other hand, it is difficult to believe in the possibility of a dual infection, although such an assumption in the case of our patient is difficult to completely rule out.

Due to the lack of response to antibiotics and spread of inflammatory processes in the vertebrae and paravertebral soft tissue, a suspicion of spine TB was placed. Today, it is clear that percutaneous computed tomography guided biopsy of the spine is an effective and well-evaluated procedures [35–37]. In order to confirm the diagnosis of spinal TB we have done a fine needle biopsy of the L3 vertebral body, successfully. Using direct observation of the obtained material, AFB were detected suspicious to *Mycobacterium tuberculosis*.

In the absence of severe neurological disorders in patients with spine TB, administration of antituberculous drugs is the first choice of therapy [38]. The appropriate duration of drug administration, as well as possible combinations of antituberculous drugs has not been precisely defined, although there are recommendations. A treatment decision should be individualized for each patient. According to the recommendations of the US Centers for Disease Control and Prevention, the Infectious Diseases Society of America, and the American Thoracic Society [39], a four drug regimen should be empirically used to treat spine TB. Isoniazid and rifampin should be administered during the whole course of therapy, and additional drugs should be administered during the first 2 months of therapy. These drugs are generally chosen among the first-line drugs [38–40]. Studies performed by the British Medical Research Council indicate that TB of the thoracolumbar spine should be treated with combination chemotherapy for 6–9 months. Regarding therapy duration, these studies did not include patients with multiple vertebral involvement, cervical lesions, or major neurologic involvement. Because of these limitations, many experts still recommend chemotherapy for 9–12 months [13, 17, 29, 32, 41].

Similar to other authors we started the treatment of our patient with a combination of four first-line antituberculous drugs in order to minimize the risk of drug resistance developing [3, 8, 17, 18, 33, 38]. For technical reasons, we started treatment with streptomycin, although the modern therapy of a specific process usually begins with combination including pyrazinamide. Five weeks later, the therapy was continued with the combination of isoniazid, rifampicin, and ethambutol, and lasted for 18 months. Good and fast effect of tuberculostatics further confirmed the diagnosis of subdural tuberculous abscess of the spine. Due to the delayed diagnosis and advanced disease, the treatment was carried out more than the recommended 9–12 months.

When subdural tuberculous abscess of the spine is diagnosed, a patient must be under constant surveillance by the infectious disease specialist and neurosurgeon with the regular consultation of radiologists. This is necessary in case of the need for early decompression of the spinal cord. This surgical procedure may lead to rapid and complete recovery of neurological deficit. However, according to the literature in most cases the treatment of spine TB passes without surgery. Neurosurgical or orthopedic treatment is required in approximately 20% of all cases of spine TB [31, 32, 37, 40, 41].

Conclusion

Subdural tuberculous abscess of spine is extremely rare manifestation of spine TB. The exact and early diagnosis and adequate treatment for atypical form of spine TB are key factors of good prognosis. We believe that this review will improve the diagnosis of spine TB in early stage of the disease, before the development of irreversible neurological deficits and spinal deformity.

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Received on May 12, 2011.
Revised on October 12, 2011.
Accepted on November 17, 2011.

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Poziv za reklamiranje u 2013. 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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P R I K A Z K N J I G E

Kako se pišu i publikuju saopštenja o biomedicinskim istraživanjima

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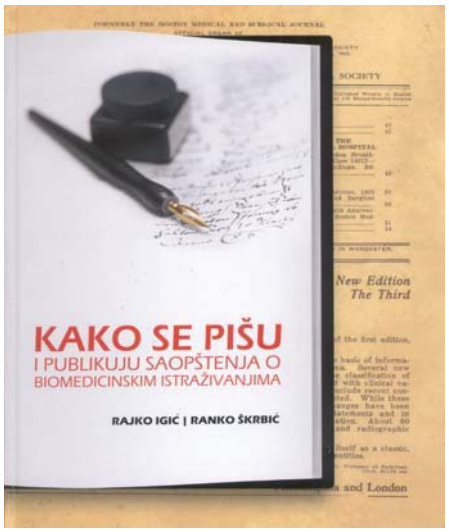
Grafomark, Laktaši, Republika Srpska, BiH
Mesto i godina izdanja:
Banja Luka, Republika Srpska, BiH, 2012.
Štampa:
Grafomark, Laktaši, Republika Srpska, BiH
Tiraž:
1000
Povez:
Tvrdi; tabaci šiveni koncem

ISBN 978-99955-57-64-5



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Knjiga „Kako se pišu i publikuju saopštenja o biomedicinskim istraživanjima“ prof. dr Rajka Igića i prof. dr Ranka Škrbića predstavlja svojevrsni vodič koji vas, korak po korak, vodi ka tom cilju. Kako se iz naslova knjige vidi, ona je pisana za istraživače iz oblasti biomedicinskih nauka, mada u njoj korisne savete mogu naći autori i iz drugih naučnih oblasti jer metodologija pripreme naučnog saopštenje je u mnogim elementima jedinstvena, bez obzira na razlike u pojedinim naučnim poljima. Iako je sadržaj knjige uglavnom usmeren ka autorima, korisne savete u njoj mogu naći i urednici/izdavači časopisa, recenzenti, ali i mentori master i doktorskih teza, dakle svi oni koji učestvuju u realizaciji naučnoistraživačkog rada.

Knjiga je podeljena u sedam poglavlja, iza kojih sledi popis korišćene literature, zatim deo Prilozi u kome su dati veoma korisni primeri u vezi sa pripremom naučnih publikacija (npr. primeri pojedinih vrsta naučnih članaka iz biomedicinskih časopisa, uključujući i prikaze knjiga, zatim članci posvećeni publikovanju u recenziranim časopisima i načinu pripreme projekta doktorske disertacije, pa primer teksta recenzije naučnog članka i odgovora autora na primedbe i sugestije recenzenta, kao i primer molbe autoru članka za dozvolu da se deo njegovog rada koristi u knjizi) i, na kraju, veoma bogat predmetni indeks, sa brojem stranice na kojoj se određeni pojam nalazi, što značajno olakšava korišćenje knjige. Pored toga, na početku knjige dat je spisak slika i tabela koji sadrže niz korisnih primera sa oznakom stranice na kojoj se nalaze, što dodatno pomaže u nalaženju potrebne informacije.

U prvom poglavlju, „Biomedicinske publikacije i izveštaji“, dat je kratak prikaz pojedinih vrsta publikacija koje se najčešće sreću u oblasti biomedicine. Budući da su primeri nekih od navedenih publikacija (npr. Pregledni članak, Pismo uredniku, Esej, Prikaz knjige) dati posle u sekciji Prilozi, trebalo je već na ovom mestu uputiti čitaoca na njih jer bi na taj način, uz data objašnjenja, odmah i video kako ta vrsta publikacije i izgleda.

Drugo poglavlje, pod naslovom „Izbor teme za istraživanje“, obrađuje najvažnije aspekte planiranja naučnog projekta, prikupljanja literature i, kao posebno značajno, upućuje čitaoca na to kako treba čitati naučni članak da bi iz nje-

govog sadržaja za što kraće vreme došao do najvećeg broja relevantnih podataka.

Treće i četvrto poglavlje, „Rukopis članka o istraživanju“ i „Priprema rukopisa“, čine okosnicu cele knjige. U njima je detaljno objašnjena struktura različitih kategorija naučnih članaka i načina kako bi ih trebalo koncipirati i napisati sa brojnim primerima iz prakse, uključujući i razmatranja jezika i stila rukopisa na srpskom i engleskom jeziku, skraćenica, upotrebe glagolskih vremena u naučnom članku, transliteraciji ćirilićnih slova u latinična, itd, tako da ove delove knjige s pravom možemo nazvati ’Bukvarom za pisanje naučnog članka’. U njima se, takođe, razmatraju i pojedine kategorije naučnih članaka, čiji su primeri dati u sekciji „Prilozi“, ali, opet, autori su propustili priliku da to navedu u tekstu i da, na taj način, primerom pokažu kako taj članak i izgleda kada se objavi u biomedicinskom časopisu.

Poglavlje „Uređivanje i publikovanje biomedicinskih časopisa“ namenjeno je, pre svega, urednicima i recenzentima, kao i izdavačima biomedicinskih časopisa, ali od značaja je i za autore jer im daje uvid u proces rada uredništva i način na koji recenzenti procenjuju njihov rad.

Deo ovog poglavlja posvećen je i radovima za sticanje akademskog zvanja (stepen magistar ili, po novom, master, i doktor nauka) sa objašnjenjima što ti radovi treba da sadrže i kako ih pripremiti, a, takođe, data su kratka uputstva o načinu kako od doktorske disertacije pripremiti članak za časopis. Dat je osvrt i na pripremu apstrakta prezentacije na naučnom skupu, kao i na pripremu postera, što može da bude od koristi mladim istraživačima koji u početku svoje karijere najčešće na ovaj način saopštavaju rezultate svojih istraživanja.

Potpoglavlje „Autorsko pravo, patentiranje i etika istraživača“ u najkraćim crtama donosi najosnovnije podatke u vezi sa etikom u naučnoistraživačkom radu, uključujući i etiku u publikovanju. Iako su u uvodnoj reči autori naveli da ovoj temi nisu posvetili veću pažnju imajući u vidu da se danas, u vreme Interneta i olakšane dostupnosti bazama naučne publicistike, moguće zloupotrebe u publikovanju naučnih radova lako otkrivaju, mišljenja sam da naši autori, kojima je ova knjiga i namenjena, još uvek ne sagledavaju ovaj problem u pravom svetlu i da mu je trebalo posvetiti više prostora u knjizi. Naime, kao urednik „Vojnosanitetskog pregleda“ vrlo često sam svedok grubog kršenja etike publikovanja od strane pojedinih autora. Najčešće se radi o slučajevima autoplagijarizma, kada autori svoj ranije objavljen rad u nekom domaćem časopisu, koji nije indeksiran u poznatim citatnim bazama, neznatno prepravljen predaju kao potpuno novi rad našem časopisu. Iz razgovora sa nekima od njih, stiče se utisak da ponekad to čine zbog neznanja kada i u kom obimu već objavljene rezultate mogu koristiti u novom radu, a kada ne. Nadam se da će u nekom od novih, dopunjenih izdanja ove knjige, čiji će tiraž, verujem, veoma brzo biti rasprodat, autori i ovom problemu posvetiti više pažnje.

Poslednja dva poglavlja u ovoj knjizi posvećena su kliničkim istraživanjima i prikazu toksikoloških rezultata na životinjama. Ukratko su navedene karakteristike pojedinih vrsta kliničkih studija s osvrtom na statističku obradu rezultata tih studija i njihov prikaz u biomedicinskim publikacijama (poglavlje „Klinička istraživanja“) i opisano je na koji način se najčešće prikazuju rezultati istraživanja u eksperimentalnoj toksikologiji (poglavlje „Prikaz toksikoloških rezultata dobijenih na životinjama“).

Do sada je na ovu temu na našem govornom području objavljeno tek nekoliko knjiga, ali ni izdaleka ne sadrže toliko praktičnih saveta i primera, kao ova knjiga. To ne treba da čudi, ako se ima u vidu da su njeni autori naučnici i univerzitetski profesori sa bogatim istraživačkim i publicističkim opusom. Profesor Igić je trenutno i glavni i odgovorni urednik međunarodnog časopisa „Scripta Medica“, a, inače, poznat je u naučnim krugovima i kao organizator brojnih, veoma uspešnih seminara na temu publikovanja u biomedicinskim časopisima, na kojima mladim istraživačima prenosi ogromno znanje i iskustvo jednog plodnog istraživača, autora, recenzenta, urednika i mentora.

Tekst je pisan lepim, ujednačenim i jasnim stilom, tako da se knjiga „lako“ čita. Iako je ona prvenstveno namenjena mladim istraživačima koji čine prve korake u svetu naučne publicistike, korisne podatke u njoj mogu naći svi koji na bilo koji način imaju veze sa publikovanjem naučnih radova, uključujući i urednike biomedicinskih časopisa i njihove recenzente. Konačno, knjigu bi trebalo da imaju i svi nastavnici koji predaju na fakultetima iz medicinskog naučnog polja jer su mnogi od njih često mentori mladim istraživačima, odnosno oni koji treba da ih uvedu u svet nauke i naučne publicistike.

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

3. ***Tekst članka***

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

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost etičkog komiteta.

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

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

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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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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.

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

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