VOLUMEN/VOLUME 33 • BROJ/NUMBER 75 • STRANE/PAGES 1676-1740• JUN/JUNE 2017

ACTA STOMATOLOGICA NAISSI

Zvanični časopis Univerziteta u Nišu, Medicinskog fakulteta i Klinike za stomatologiju

Offical publication of the University of Niš, Faculty of Medicine and Clinic of Dentistry

YU-ISSN 0352-5252

UDK.616.31 (05)(06)(479.11)

Acta Stomatologica NAISSI

ASN YU-ISSN-0352-5252
UDK.616.31(05)(06)(479.11)

ZVANIČNA PUBLIKACIJA UNIVERZITETA U NIŠU,
MEDICINSKOG FAKULTETA
I KLINIKE ZA STOMATOLOGIJU

OFFICIAL PUBLICATION OF THE UNIVERSITY OF NIŠ,
FACULTY OF MEDICINE AND
CLINIC OF DENTISTRY

ISSN (electronic version) 1820-1202

ČASOPIS INDEKSIRAN U *ONLINE* BIBLIOTECI
INDEXED IN *ONLINE* LIBRARY

EBSCO - http://ejournals.ebsco.com
DOAJ - www.doaj.org
SCOPUS & EMcare - www.elsevier.com i www.info.scopus.com

ADRESA UREDNIŠTVA / ADRESS OF THE EDITORIAL BOARD

Acta Stomatologica Naissi, Klinika za stomatologiju, Bulevar dr Zorana Đinđića 52, 18000 Niš, Srbija
www.medfak.ni.ac.rs /ASN/
Acta Stomatologica Naissi, Clinic of Dentistry, Bulevar dr Zorana Đinđića 52, 18000 Niš, Srbija
www.medfak.ni.ac.rs /ASN/
Telefon/Phone: +381 (0)18 453 86 55

Časopis se štampa dva puta godišnje u junu i decembru. Pretplata za 2014. godinu je 5000,00 dinara za fizička lica, a za ustanove 10000,00 dinara. Pretplata se uplaćuje na tekući račun – Klinika za stomatologiju u Nišu br. 840-591667-33, sopstvena sredstva.
The Journal is published two times a year in June and December. Subscription for 2014. is 50 € for individuals, and 100€ for institutions. For detailes of payment contact: e-mail: tijanicm@yahoo.com.

Časopis finansiraju / The Journal is financially supported by:
Ministarstvo nauke Republike Srbije
(rešenje br. 451-03-2489/2002-02; 451-03-330/2003-02; 451-03-884/2004-02; 451-03-4180/2005-02;451-03-286/2009/2010-02/1; 451-03-1143/2011-14-2)
Medicinski fakultet i Klinika za stomatologiju u Nišu.
Ministry of Science Republic of Serbia
(decision # 451-03-2489/2002-02; 451-03-330/2003-02; 451-03-884/2004-02; 451-03-4180/2005-02;451-03-286/2009/2010-02/1; 451-03-1143/2011-14-2)
Faculty of Medicine and Clinic of Dentistry Nis

ŠTAMPA / PUBLISHER

GALAKSIJA,NIŠ

ELSEVIER

Bibliographic Databases

Univerzitet U Nisu
Medicinski Fakultet
Clinic of Stomatology
Dr. Zoran Đinđić Boulevard 81
Nis 18000
Serbia

12 February 2009

Re: Complimentary Subscription Request
Acta Stomatologica Naissi will be covered by **Scopus & EMCare**

Dear Sir/ Madam,

In recognition of the high quality and relevance to the scientific community of the journal listed, we are pleased to inform you that your publication has been selected for coverage in the Elsevier Bibliographic Databases indicated above as of **2009**.

We would like to request a complimentary subscription so that we may properly index this title. As you will no doubt already know, inclusion in abstract & indexing (A&I) databases increases the visibility and awareness of your full-text journals. The A&I databases will drive usage and traffic to your full-text platforms with sophisticated linking technologies, increasing journal brand awareness and subscription sales.

Since efficient coverage and rapid record processing enhance the value of any database – increasing its benefits to you as a publisher – we would like to ask your cooperation in granting us online access to the publication website http://www.medfak.ni.ac.yu/ASN/index.htm.

Our databases include standard A&I record components. Please be assured that we do not make any full text available or provide content to any third parties.

After consideration and upon agreement, we would appreciate it if you could complete and return the attached complimentary subscription acknowledgment form by April 1st, 2009 or alternatively inform us of the access details – should these be required - via email (bd-scm@elsevier.com).

Please feel free to contact me for additional information or to address any questions that you may have. Thank you in advance for your cooperation.

Sincerely,
Jaqui Mason (Ms),
Vice President, BD Operations

Elsevier Bibliographic Databases *produces a range of leading abstract & indexing databases, which scans the world's STM serials literature for coverage across all science and social sciences disciplines. Databases include Scopus, EMBASE, EMCare, Compendex, GEOBASE, Elsevier BIOBASE, FLUIDEX, World Textiles and several specialized niche databases and other derivative products such as Mosby Yearbooks.*
Scopus*, the largest abstract and citation database of research literature and quality web sources, was created for researchers to help them find the quality information they need from around the globe. Since its introduction in 2004, Scopus has developed a solid customer base of over 1000 institutional customers worldwide enabling millions of users to make their literature research process more effective and productive.*

For an overview and details of Elsevier Bibliographic Databases, please visit www.elsevier.com (click on Bibliographic databases) and www.info.scopus.com

Acta Stomatologica NAISSI

Jun/ June. 2017, Vol. 33, br./ num. 75str./p 1676-1740

SADRŽAJ / CONTENTS

OBAVEŠTENJE

Od 1. januara 2012. godine Acta Stomatologica Naissi je u sistemu ELEKTRONSKOG UREĐIVANJA ČASOPISA (e-Ur) .

Autori, recenzenti, urednici, menadžeri i ostali, kao potencijalni korisnici našeg časopisa, moraju biti registrovani sa e-mail adresom. Registraciju je moguće izvršiti na adresi:

http://scindeks-eur.ceon.rs/index.php/asn

NOTICE

As of January 1st, 2012 the journal Acta Stomatologica Naissi is in the system ELECTRONIC JOURNAL EDITING (e-Ur).

The authors, reviewers, editors, managers and others, as potential beneficiaries of the Journal, must be registered with the e-mail address. Registration can be made at:

http://scindeks-eur.ceon.rs/index.php/asn

Primljen/ Recived on : 01.03.2017
Revidiran/ Revised on : 23.03.2017
Prihvaćen/ Accepted on : 21.04.2017

ORGINALNI RAD
KLINIČKA STUDIJA
ORIGINAL ARTICLE
CLINICAL STUDY
doi:10.5937/asn1775676Z

IMUNOHISTOHEMIJSKE I KARIOMETRIJSKE RAZLIKE I SLIČNOSTI TUMORA PLJUVAČNIH ŽLEZDI IZMEĐU PLEOMORFNOG ADENOMA, ADENOMA BAZALNIH ĆELIJA I POLIMORFNOG ADENOKARCINOMA NISKOG GRADUSA

IMMUNOHISTOCHEMICAL AND KARYOMETRIC SIMILARITIES AND DIFFERENCES OF SALIVARY GLAND TUMORS BETWEEN PLEOMORPHIC ADENOMA, BASAL CELL ADENOMA AND POLYMORPHOUS LOW GRADE ADENOCARCINOMA

Nikola D. Živković [1,2], Dragan S. Mihailović[1,2], Miloš S. Kostić[1,3], Ana S. Cvetanović[1,4], Tijana V. Denčić[1,2], Maja V. Jovičić Milentijević[1,2], Žaklina Ž. Mijović[1,2], Simona M. Stojanović [2], Milan S. Spasić[5], Zoran U. Pešić[2,6], Miloš N.Trajković[6]

[1]CENTAR ZA PATOLOGIJU I PATOLOŠKU ANATOMIJU, KLINIČKI CENAR, NIŠ, SRBIJA
[2]UNIVERZITET U NIŠU, MEDICINSKI FAKULTET, SRBIJA
[3]UNIVERZITET U NIŠU, INSTITUT ZA IMUNOLOGIJU, MEDICINSKI FAKULTET, SRBIJA
[4]KLINIKA ZA ONKOLOGJU, KLINIČKI CENAR, NIŠ, SRBIJA
[5]ODELJENJE ZA ORALNU HIRURGIJU, KLINIKA ZA STOMATOLOGIJU, NIŠ, SRBIJA
[6] ODELJENJE ZA MAKSILOFACIJALNU HIRURGIJU, KLINIKA ZA STOMATOLOGIJU, NIŠ, SRBIJA

[1]CENTER FOR PATHOLOGY AND PATHOLOGICAL ANATOMY, CLINICAL CENTER NIŠ, NIŠ, SERBIA
[2]UNIVERSITY OF NIŠ, FACULTY OF MEDICINE, NIŠ, SERBIA
[3] UNIVERSITY OF NIŠ, FACULTY OF MEDICINE, INSTITUTE OF IMMUNOLOGY, NIŠ, SERBIA
[4]ONCOLOGY CLINIC, CLINICAL CENTER NIŠ, NIŠ, SERBIA
[5]DEPARTMENT OF ORAL SURGERY, CLINIC OF DENTISTRY, NIŠ, SERBIA
[6]DEPARTMENT OF MAXILLOFACIAL SURGERY, CLINIC OF DENTISTRY, NIŠ, SERBIA

Sažetak

Uvod: *Tumori pljuvačnih žlezda su veoma retke neoplazme. S obzirom na njihovu patohistološku sliku, ovi tumori predstavljaju veoma veliki dijagnostički izazov.*
Cilj: *istaživanja je diferencijacija ova tri tipa tumora primenom imunohistohemijske i morfometrijske analize, kao i određivanje visine Ki67 proliferativnog indeksa.*
Materijal i metode: *Istraživanje je obuhvatilo 44 tumora, 20 pleomorfnih adenoma, 12 adenoma bazalnih ćelija i 12 polimorfnih adenokarcinoma niskog gradusa. Analizirana je ekspresija Ki67, p53 i HER-2 antigena, kao markera proliferacije. U sklopu diferencijalne dijagnostike, analizirana je ekspresija CEA, EMA, GFAP, p63, vimentina, CK14, α-SMA, S-100 protein i WT1 antigena. Morfometrijska analiza vršena je u softverskom paketu „ImageJ" verzija 1.43u.*
Rezultati: *Neoplastične ćelije u pleomorfnom adenomu su pokazale jaku ekspresiju GFAP, p63, WT1, vimentin i S100. U grupi od dvanaest polimorfnih adenokarcinoma niskog gradusa prisutna je difuzna ekspresija CK14, S100, vimentin i EMA su bili apsolutno eksprimirani, dok je αSMA bio negativan. Adenom bazalnih ćelija pokazuje pozitivnost na S-100, CEA, p63 i vimentin. Analizom vrednosti proliferativnog Ki67 indeksa ustanovljena je statistički značajna razlika u grupi pleomorfnog adenoma, što se dovodi u vezu sa čestim recidiviranjem. Morfometrijskom analizom se uočavaju veće vrednosti u grupi polimorfnog adenokarcinoma niskog gradusa, ali su statistički značajne razlike nađene samo za Feretov dijametar i integrisanu optičku gustinu u odnosu na pleomorfni adenom ($p<0,05$). U grupi adenoma bazalnih ćelija tumorske ćelije su pokazale statistički veće vrednosti za integrisanu optičku gustinu u odnosu na pleomorfni adenom ($p<0,001$).*
Zaključak: *Za diferencijalnu dijagnozu tumora pljuvačnih žlezda, pored osnovne mikromorfološke, neophodna je i imunohistohemijska i morfometrijska analiza.*

Ključne riječi: pleomorfni adenom, polimorfni adenokarcinom niskog gradusa, adenom bazalnih ćelija, imunohistohemija, morfometrija

Address for correspondence:
Asist. dr sci. med. Nikola D. Živković
nikolazivkovich@gmail.com
0631045645

Abstract

Introduction: *Salivary gland tumors are extremely rare neoplasms. Given their pathohistological image, these tumors represent a great diagnostic challenge.*
The aim *of our study was to differentiate these three tumor types by applying the immunohistochemical and morphometric analysis.*
Material and Methods: *The entire study was conducted at the Center for Pathology and Pathological Anatomy in Niš. The study included 44 tumors, 20 pleomorphic adenomas, 12 basal cells adenomas and 12 polymorphous low-grade adenocarcinomas. The expression of Ki67, p53 and HER-2 antigens, as proliferation markers, was analyzed. The differential diagnostics also included the analysis of the expression of CEA, EMA, GFAP, p63, vimentin, CK14, α-SMA, S-100 protein and WT1 antigen. The morphometric analysis was done in the program pack "ImageJ" version 1.43u. The statistical analysis of data was done in the program pack SPSS 15.0.*
Results: *Neoplastic cells in pleomorphic adenoma showed a strong expression of GFAP (20/20), p63 (20/20), WT1 (20/20), vimentin (18/20) and S100 (16/20). Diffuse expression of CK14 (12/12) was present in the group of 12 polymorphous low-grade adenocarcinomas. S-100, vimentin and EMA were absolutely expressed, whereas α-SMA was negative. Basal cell adenoma showed negativity to S-100, CEA, p63 and vimentin. The analysis of the proliferative Ki67 index values pointed to a statistically significant difference in the pleomorphic adenoma group, which was associated with a frequent recurrence of this benign tumor. The analysis of morphometric characteristics showed higher values in the polymorphous low-grade adenocarcinoma group, but statistically significant differences were found only for the Feret diameter and the integrated optical density ($p < 0.05$). As for the basal cell adenoma group, tumor cells showed statistically higher values for the integrated optical density ($p < 0.001$).*
Conclusion: *Apart from the basic micromorphological analysis, the differential analysis of salivary gland tumors also requires the immunohistochemical analysis as well as the monitoring of morphometric characteristics of these tumors' nuclei.*

Key words: pleomorphic adenoma, polymorphous low-grade adenoma, basal cell adenoma, immunohistochemistry, morphometry

Uvod

Tumori pljuvačnih žlezda su veoma retke neoplazme. S obzirom na njihovu patohistološku sliku, ovi tumori predstavljaju veoma veliki dijagnostički izazov. Učestalost ovih tumora se kreće od 3 do 6% svih tumora regije glave i vrata [1,2]. Parotidna žlezda je najčešće mesto tumora pljuvačnih žlezda, sa učestalošću od 80-85%, a skoro 75% ovih tumora su benigne neoplazme. Druga po učestalosti je submandibularna žlezda, sa 10%, ali polovina tumora ove lokalizacije su maligne neoplazme. Sa frekvencijom od 1% je sublingvalna žlezda, ali oko 80% tumora je maligno. Male pljuvačne žlezde su mesta sa najčešćom frekvencijom malignih tumora [3,4]. Epidemiološki podaci prikazuju različitu učestalost u različitim etničkim grupama i delovima sveta, što dodatno otežava globalnu incidenciju ovih tumora [5,6]. Prosečna starost bolesnika je 46-47 godina, ali pik pojedinih tumora je u šestoj i sedmoj deceniji života [2].

Pleomorfni adenom, označen još i kao mešoviti tumor (tumor mixtus), predstavlja najčešću neoplazmu pljuvačnih žlezda. Sreće se u svim životnim dobima, ali pik incidencije je u petoj deceniji. Jednako je zastupljen među polovima, sa blagom dominacijom kod žena. U 80% slučajeva lokalizovan je u parotidnoj žlezdi, i to u donjem polu. Ako je lokalizovan u dubokom režnju, ima prezentaciju parafaringealnog tumora; obično su sporog rasta i bezbolni su. Mali tumori se prezentuju kao glatke, čvrste, mobilne globule, dok tumori većih dimenzija mogu oštetiti kožu i sluzokožu koja ih prepokriva. Multifokalni i rekurentni tumori mogu biti fiksirani za okolno tkivo. Sporadično se mogu javiti sa drugim tumorima, naročito sa Warthin tumorom. Veličina tumora varira od 2 do 5 cm, ali mogu dostići i enormne dijametre. U slučajevima kada se javi infarceracija tumora, mogu se javiti bol i parestezije. Na nepcu se najčešće javljaju na prelazu između mekog i tvrdog nepca, kada mogu biti fiksirani, zbog blizine periosta [7,8].

Makroskopski su jasno definisani, okruglog do ovalnog oblika. Debljina kapsule varira, a može i nedostajati, naročito u manjim, mukusnim žlezdama. Ponekad mogu da probiju samu kapsulu i da daju izgled formiranja novog tumora, koji se prezentuje kao satelitski nodus; međutim, uvek je u kontaktu sa tumorom. Spoljašnja površina tumora je lobulirana, a na preseku su homogenog izgleda, uglavnom belo prebojeni. Kada je reč o tumoru sa obilnom hrskavičavom ili miksohondroidnom stromom, imaju sedefasti sjaj.

Introduction

Salivary gland tumors are extremely rare neoplasms. Given their pathological image, these tumors represent a great diagnostic challenge. The incidence of these tumors ranges from 3-6% of all tumors of the neck and head region [1,2]. The parotid gland is the most common site of salivary tumor glands, with the incidence of 80-85%, and almost 75% of these tumors are benign neoplasms. The second most frequent site is the submandibular gland with 10%, but one half of tumors of this localization is malignant neoplasms. The sublingual gland is affected in 1% of cases, however, about 80% of tumors are malignant. Minor salivary glands are the sites with the highest incidence of malignant tumors [3,4]. Epidemiological data show a different incidence in different ethnic groups and parts of the world, which further complicates the global incidence of these tumors [5,6]. The mean age of patients is 46-47 years, but the peak of some tumors is in the sixth and seventh decade of life [2].

Pleomorphic adenoma, also referred to as mixed tumor (tumor mixtus), is the most common salivary gland neoplasm. It occurs at all ages, but the peak of incidence is in the fifth decade of life. It is equally represented among the sexes, with a slight domination in women. In 80% of cases, it is localized in the lower pole of the parotid gland. If localized in the deep cortex, it has a presentation of parapharyngeal tumor; they are usually of slow growth and painless. Minor tumors are presented as smooth, solid, mobile globules, whereas large tumors can damage the skin and mucosa which covers them. Multifocal and recurrent tumors may be attached to the surrounding tissue. They may sporadically appear with other tumors, especially with Warthin's tumor. The tumor size varies from 2 to 5 cm, however, they may reach enormous diameters as well. Pain and paresthesia may occur in cases of tumor incarceration. When it comes to the palate, they occur usually on the transition between the soft and hard palate, when they can be attached, due to the proximity of periosteum [7,8].

They are clearly defined macroscopically and have a round to oval shape. The capsule thickness varies, or even lacks, especially in smaller, mucous glands. Sometimes, they can break the capsule itself and appear as if a new tumor was forming, which is presented as a

Patohistološki, tumor je izgrađen od epitelne, mioepitelne i mezenhimne komponente, koja može biti mukoidnog, miksoidnog ili hondroidnog izgleda. Epitelna komponenta formira plaže ili strukture nalik duktusima, a same ćelije mogu biti kuboidalne, vretenaste, plazmocitoidne, skvamozne i ćelije svetle citoplazme. Ponekad epitelna komponenta može biti predominantna u tumoru, što je označeno kao celularni pleomorfni adenom, ali je bez prognostičkog značaja. Duktuse čine luminalne, kuboidalne ćelije, a mogu da imaju i abluminalni sloj mioepitelnih ćelija. Luminalne ćelije, ponekad, imaju svetlu citoplazmu i hiperhromna jedra, što može da zada veliki diferencijalno-dijagnostički problem ka adenoidno-cističnom ili epitelno-mioepitelnom karcinomu. Mezenhimna komponenta tumora je mukoidna, miksoidna, kartilaginozna ili hijalina. i ona može da čini dominantnu komponentu. Ćelije sa mukoidnom supstancom su zapravo mioepitelne ćelije. Ovde mogu da se vide i područja koštane metaplazije. Višegodišnji tumori pokazuju izraženu hijalinizaciju, u tolikoj meri da je epitelna komponenta prisutna u tragovima i sa znacima degeneracije. Takvi tumori predstavljaju veliki rizik za malignu transformaciju [8-10].

Imunohistohemijski, epitelne duktalne ćelije su pozitivne na EMA, CEA GFAP, CK14, dok je mioepitelna komponenta pozitivna na αSMA, p63, vimentin, S100 i GFAP. Poslednjih godina je uočena pozitivna reakcija sa WT1. Naime, modifikovane mioepitelne ćelije pokazuju izrazitu citoplazmatsku ekspresiju proteina [7].

Adenom bazalnih ćelija predstavlja retku benignu neoplazmu pljuvačnih žlezda, bazaloidnog fenotipa. Generalno, sreće se u 1-3% slučajeva svih tumora pljuvačnih žlezda, sa pikom u sedmoj deceniji. Među polovima je češći kod žena, sa odnosom 2:1. Najčešće je lokalizovan u velikim pljuvačnim žlezdama, i to u parotidnoj. Klinički se prezentuje kao jasno ograničeni, pokretni nodus, čvrste konzistencije [7,11,12].

Makroskopski, prezentuju se kao tumori sa kapsulom, solidne do cistične građe, belosive do braon prebojenosti, veličine 1-3 cm. Membranozni tip može biti multinodularan ili multifokalan.

Patohistološki, tumor je izgrađen od bazaloidnih ćelija, nejasnih međućelijskih granica, svetle citoplazme sa ovalnim do okruglim jedrima. Ćelije formiraju solidne, trabekularne ili tubularne formacije.

satellite node. However, it is always in contact with the tumor. The outer surface of the tumor is lobular, it is homogenous at the intersection, and mostly white in color. When it comes to tumors with abundant cartilaginous or mixochondroid stroma, they have a pearly gloss.

Pathohistologically, the tumor is comprised of the epithelial, myoepithelial and mesenchymal component which can have mucoid, myxoid or chondroid appearance. The epithelial component forms nests or duct-like structures, and the cells themselves can be cuboidal, spindle-shaped, plasmocytoid, squamous or light cytoplasm cells. The epithelial component can sometimes be predominant in tumors, which are in such cases labelled as cellular pleomorphic adenomas, but it has no prognostic significance. Ducts are comprised of luminal, cuboidal cells and they can also have the abluminal layer of myoepithelial cells. Luminal cells sometimes have light cytoplasm and hyperchromic nuclei, which can be a great problem in the differential diagnosis of adenoid cystic or epithelial-myoepithelial carcinoma. The mesenchymal component of the tumor is mucoid, myxoid, cartilaginous or hyaline, and it can be the dominant component as well. Cells with mucoid substance are actually myoepithelial cells. Areas of bone metaplasia can also be seen here. Perennial tumors show a pronounced hyalinization, such that the epithelial component is present in traces and with signs of degeneration. Such tumors represent a great risk for malignant transformation [8-10].

Immunohistochemically, ductal epithelial cells are positive to EMA, CEA GFAP, CK14, whereas the myoepithelial component is positive to αSMA, p63, vimentin, S100 and GFAP. A positive reaction with WT1 has been noticed in recent years. Namely, modified epithelial cells exhibit a pronounced cytoplasmic expression of proteins [7].

Basal cell adenoma is a rare benign salivary gland neoplasm of basaloid phenotype. In general, it is found in 1-3% of cases of all salivary gland tumors, with the peak in the seventh decade of life. It is more frequent in women, with a ratio of 2:1. It is usually localized in large salivary glands, especially in the parotid gland. In terms of clinical presentation, it is a clearly circumscribed, mobile node of solid consistency [7,11,12].

Macroscopically, they are presented as encapsulated tumors, of solid to cystic structuire, white-gray to brown-colored, 1-3 cm in size. The membranous type can be multinodular or multifocal.

Kod solidnog tipa rasta, tumorske plaže su različitog oblika sa perifernim radijalnim ćelijskim rasporedom (eng. palisading), međusobno odvojene gustim snopovima kolagenih vlakana. Trabekularni tip se odlikuje bazaloidnim ćelijama koje formiraju uske trake ili trabekule, odvojene vaskularnom stromom. Kod tubularnog tipa dominiraju duktalne strukture. Membranozni tip se karakteriše širokim trakama hijalinog veziva, po periferiji tumorskih plaža, kao i intracitoplazmatskim inkluzijama.

Imunohistohemijski, ćelije na periferiji tumorskih plaža eksprimuju p63, vimentin, SMA i S100, dok su luminalne ćelije pozitivne na CK14, CEA i S100. Analizirajući ekspresiju markera, dolazimo do zaključka da je adenom bazalnih ćelija, histogenetski, poreklom od ćelija interkalatnih kanala [7].

U svakoj varijanti adenoma mogu se videti cistične strukture, skvamozna i onkocitna (u tubularnom tipu) diferencijacija, kao i kribriformni rast [7,13,14].

Polimorfni adenokarcinom niskog gradusa predstavlja primarni maligni epitelni tumor pljuvačnih žlezda sa veoma polimorfnom prezentacijom, monomorfnim ćelijama, infiltrativnim rastom i niskim metastatskim potencijalom. Učestalost tumora iznosi 26% svih intraoralnih karcinoma. Zastupljeniji je u nešto starijoj poulaciji (oko 70% bolesnika je od 50 do 70 godina starosti), sa predominacijom kod žena, i to u odnosu 2:1. U oko 60% slučajeva je lokalizovan na nepcu, potom na bukalnoj sluzokoži, retromolarno, gornjoj usni i podu jezika. Retko je prisutan u velikim pljuvačnim žlezdama. Prezentuje se u vidu bezbolne mase, koja može da bude praćena krvarenjem, telangiektazijama i ulceracijama [7,13].

Makroskopski, tumor je jasno ograničen, ali bez kapsule. Čvrste je konzistencije, prljavo žute prebojenosti, lobuliranog izgleda.

Patohistološki, tumor je izgrađen od monomorfnih ćelija, male do srednje veličine sa malim, ovalnim i hiperhromnim jedrima, baz jasno uočljivih jedaraca. Mitoze i nekroze su retke. Pod polimorfizmom u samom nazivu ovog tumora podrazumeva se solidni, kribriformni, tubularni, trabekularni, fascikularni (eng. streaming), linearni (eng. indian file) i cistični tip rasta. Uočava se targetoidna perineuralna i perivaskularna invazija [7,15].

Stroma može da bude hijalinizovana, mukoidna ili fibrozna, što može dodatno komlikovati diferencijalnu dijagnozu.

Pathohistologically, the tumor is composed of the basaloid cells of blurry intercellular boundaries, with light cytoplasm with oval to round nuclei. The cells form solid, trabecular or tubular formations. In the solid growth type, tumor nests are of different shape with peripheral radial cell arrangement (palisading), separated from each other by thick bundles of collagen fibers. The trabecular type is characterized by the basaloid cells which form narrow strips or trabeculae, separated by the vascular stroma. In the tubular type, ductal structures are dominant. The membranous type is characterized by wide strips of hyaline binder at the periphery of tumor nests, as well as intracytoplasmic inclusions.

Immunohistochemically, the cells at the periphery of tumor beaches express p63, vimentin, SMA and S100, whereas luminal cells are positive to CK14, CEA and S100. Having analyzed the expression of markers, we concluded that basal cell adenoma was histogenetically of intercalated channel cells origin [7].

Cystic structures, squamous and oncocytic (in the tubular type) differentiation, as well as the cribriform growth, can be seen in each variant of adenoma [7,13,14].

Polymorphous low-grade adenocarcinoma is the primary malignant epithelial tumor of the salivary glands, with a rather polymorphous presentation, monomorphic cells, the infiltrative growth and a low metastatic potential. The incidence of the tumor is 26% of all intraoral carcinomas. It is more common in elderly population (around 70% of patients is 50-70 years of age), with a predominance in women with a 2:1 ratio. In about 60% of cases, it is localized on the palate, then on the buccal mucosa, in the retromolar area, on the upper lip and the floor of the tongue. It is rarely found in large salivary glands. It is presented in the form of a painless mass which may be accompanied by bleeding, telangiectasia and ulceration [7, 13].

Macroscopically, the tumor has clear boundaries, but without a capsule. It is of firm consistency, dirty-yellow in color, and of a lobulated appearance.

Pathohistologically, the tumor is composed of the monomorphic cells, of small to medium size, with small, oval and hyperchromatic nuclei, but without clearly visible nucleoli. Mitosis and necrosis are rare. The term polymorphous in the name of this tumor stands for a solid, cribriform, tubular, trabecular, fascicular (streaming), linear (indian file) and cystic growth type.

Imunohistohemijski, tumorske ćelije su pozitivne na CEA, EMA, vimentin, S100 i CK14, a negativne na p63, αSMA i GFAP [7].

Diferencijalno dijagnostički se uključuje pleomorfni adenom i adenoidni cistični karcinom.

Cilj

S obzirom na polimorfizam i preklapanje mikromorfološke prezentacije pleomorfnog adenoma, adenoma bazalnih ćelija i polimorfnog adenokarcinoma niskog gradusa, cilj našeg istaživanja bio je diferencijacija tri tipa tumora primenom imunohistohemijske i morfometrijske analize, kao i određivanje visine Ki67 proliferativnog indeksa.

Materijal i metode

Celokupno istraživanje je sprovedeno na Institutu za patologiju Medicinskog fakulteta u Nišu. Analizirani materijal predstavlja tkivo dobijeno operacijom i biopsijom pljuvačnih žlezda, sa Klinike za Maksilofacijalnu hirurgiju u Nišu. Odnos polova je varirao u zavisnosti od tumora, ali je prisutna blaga predominacija ženskog pola, što je izraženije sa malignim tumorima. Starost bolesnika varirala je u opsegu od 12 do 75 godina, naročito kod benignih lezija. Maligniteti u dečijem dobu nisu bili registrovani. Nakon primenjene intervencije, tkivo se fiksira u 10% formalinu, najmanje 24 h, po preporukama Američkog udruženja onkologa i koledža patologa (*eng. American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP))* [16]. Istraživanje je obuhvatilo 44 reprezentativna tumora pljuvačnih žlezda, 20 pleomorfnih adenoma, 12 adenoma bazalnih ćelija i 12 polimorfnih adenokarcinoma niskog gradusa. Od ukupno 44 pregledana materijala, 40 je bilo operativnih, a četiri su biopsijskih materijala. Detaljniji prikaz analiziranih tumora dat je u Tabeli 1. Mikroskopskom analizom dobijenih preparata vršena je preliminarna dijagnostika. Istovremeno, odeređivan je i reprezentativni isečak, što podrazumeva veličinu tumorskog polja sa minimalnim poljima nekroze i zapaljenskim infiltratom na kome su se radila imunohistohemijska bojenja. Imajući u vidu polimorfnost tumora pljuvačnih žlezda, još jedan od parametara za izbor isečka bila je i najpolimorfnija slika na patohistološkom preparatu. Kao kontrola služilo je zdravo tkivo pljuvačne žlezde.

Targetoid perineural and perivascular invasion can be seen [7, 15].

The stroma can be hyalinized, mucoid or fibrous, which can further complicate the differential diagnosis.

Immunohistochemically, tumor cells are positive to CEA, EMA, vimentin, S100 and CK14, but negative to p63, αSMA and GFAP [7].

The differential diagnosis includes pleomorphic adenoma and adenoid cystic carcinoma.

Aim

Given the polymorphism and overlapping of the micromorphological presentation of pleomorphic adenoma, basal cell adenoma and polymorphous low-grade adenocarcinoma, the aim of our study was to differentiate these three tumor types by applying the immunohistochemical and morphometric analysis.

Material and Methods

The entire study was conducted in the Center for Pathology and Pathological Anatomy in Niš. The analyzed material was a tissue obtained by the surgical procedure or biopsy of salivary glands, from the Maxillofacial Surgery Clinic in Niš. The gender ratio varied depending on the tumor, but there was a slight predominance of women, especially in cases of malignant tumors. The age of the patients also varied, ranging from 12 to 75 years, especially in benign lesions. Malignity in children was not registered. Upon the applied intervention, the tissue was fixed in 10% formalin, as recommended by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) [16]. The study included 44 representative salivary gland tumors, 20 pleomorphic adenomas, 12 basal cells adenomas and 12 polymorphous low-grade adenocarcinomas. Forty out of the total of 44 examined specimens were operational, whereas the remaining 4 were biopsy materials. A more detailed review of the analyzed tumors is presented in Table 1. The preliminary diagnostics included the microscopic analysis of the obtained preparations. Simultaneously, a representative tissue sample was determined, i.e. part of the tumor area with minimal necrosis and inflammatory infiltrate on which immunohistochemical staining had been done.

Analizirana je ekspresija Ki67, p53 i HER-2 antigena, kao marker proliferacije. U sklopu diferencijalne dijagnostike analizirana je ekspresija karcino-embrionalnog antigena, epitelnog membranskog antigena, kiselog glijalnog fibrilarnog proteina, p63 antigena, vimentina, citokeratina 14, α-glatkomišićnog aktina, S-100 protein i Vilms tumor 1 antigena. Pravljene su digitalne mikrofotografije, kako osnovnih tako i preparata dobijenih imuno-histohemijskim bojenjem.

Imunohistohemijska bojenja vršena su na isečcima debljine do 5μm, dobijenih iz parafinskih blokova. Za analizu je uziman reprezentativan isečak, a primenjena su sledeća antitela: anti-Ki67 (MiB-1, ready to use; DAKO, Glostrup, Denmark), anti-p53 (DO-7, ready to use; DAKO, Glostrup, Denmark), anti-p63 (e DAK-p63, ready to use; DAKO, Glostrup, Denmark), anti-HER-2 (HercepTest™, DAKO, Glostrup, Denmark), anti-CEA (II-7, ready to use; DAKO, Glostrup, Denmark), anti-EMA (E29, ready to use; DAKO, Glostrup, Denmark), anti-S-100 (S-100, ready to use; DAKO, Glostrup, Denmark), anti-CK14 (LL002, 1:20; Novocastra Laboratories, Newcastle, UK), anti-WT-1 (6F-H2, ready to use; DAKO, Glostrup, Denmark), anti-GFAP (6F2, ready to use; DAKO, Glostrup, Denmark), anti-αSMA (1A4, ready to use; DAKO, Glostrup, Denmark), anti-vimentin (V9, ready to use; DAKO, Glostrup, Denmark).

Za pozitivnu reakciju smatra se bojenje jedara za sledeće markere: Ki67, p53, p63 i WT1. Pozitivno membransko bojenje analizirano je za HER-2, a za CEA i EMA membransko i citoplazmatsko, a za S-100 jedarno i citoplazmatsko. Pozitivno citoplazmatsko bojenje vidi se kod αSMA, GFAP, CK14 i vimentina.

Ki67 indeks određivan je analizom i brojanjem pozitivnih jedara na 10 vidnih polja, na uveličanju x40. Indeks je izražen u procentima, kao odnos pozitivnih tumorskih ćelija u odnosu na negativne, neobojene ćelije.

Morfometrijska analiza vršena je u softverkom paketu „*ImageJ*" verzija 1.43u (public domain software, Wayne Rasband, National Institutes of Health, Bethesda, Maryland, USA). Mikrofotografije u boji dobijene su digitalnom kamerom visoke rezolucije (Nikon, DS-Fi1, Tokyo, Japan), koja je povezana sa mikroskopom (Nikon, ECLIPSE 50i, Tokyo, Japan). Nakon toga, slika je prebačena na kompatibilni računar i vršena je analiza jedarnih parametara primenom paketa.

Considering the polymorphism of salivary gland tumors, one of the parameters for the selection of tissue samples was the most polymorphous image on the patho-histological preparation. The healthy tissue of the salivary gland served as the control.

We analyzed the expression of Ki67, p53 and HER-2 antigens, as well as the expression of proliferation makers. The expression of the carcinoembryonic antigen, epithelial membrane antigen, glial fibrillary acidic protein, p63 antigen, vimentin, cytokeratin 14, α-smooth muscle actin, S-100 protein and Wilms' tumor 1 antigen was analyzed within the differential diagnosis. Digital photomicrographs of both basic and preparations obtained from immuno-histochemical staining were made.

Immunohistochemical staining was done on tissue samples of up to 5μm of thickness, obtained from paraffin blocks. A representative tissue sample was taken for the analysis, and the following antibodies were applied: anti-Ki67 (MiB-1, ready to use; DAKO, Glostrup, Denmark), anti-p53 (DO-7, ready to use; DAKO, Glostrup, Denmark), anti-p63 (e DAK-p63, ready to use; DAKO, Glostrup, Denmark), anti-HER-2 (HercepTest™, DAKO, Glostrup, Denmark), anti-CEA (II-7, ready to use; DAKO, Glostrup, Denmark), anti-EMA (E29, ready to use; DAKO, Glostrup, Denmark), anti-S-100 (S-100, ready to use; DAKO, Glostrup, Denmark), anti-CK14 (LL002, 1:20; Novocastra Laboratories, Newcastle, UK), anti-WT-1 (6F-H2, ready to use; DAKO, Glostrup, Denmark), anti-GFAP (6F2, ready to use; DAKO, Glostrup, Denmark), anti-αSMA (1A4, ready to use; DAKO, Glostrup, Denmark), anti-vimentin (V9, ready to use; DAKO, Glostrup, Denmark).

The staining of nuclei was considered positive for the following markers: Ki67, p53, p63 and WT1. Positive membrane staining was analyzed for HER-2, membrane and cytoplasmic staining for CEA and EMA, whereas nuclear and cytoplasmic staining was analyzed for S-100. Positive cytoplasmic staining could be seen in αSMA, GFAP, SK14 and vimentin.

The Ki67 index was determined by analyzing and counting the positive nuclei in 10 visible areas, at the magnification of x40. The index was expressed in percentage, as the ratio between positive tumor cells and negative, unstained cells. The morphometric analysis was done in the software pack "*ImageJ*" version 1.43 u (public domain

Osmobitna slika je manuelno obrađivana, nakon kalibracije, korišćenjem kompjuterskog miša. Analizirano je 100 nasumično odabranih tumorsko-ćelijskih jedara, na uveličanju x40, i to ćelija koje se ne preklapaju. Analizirano je šest jedarnih parametara: površina (eng. area), perimetar (*eng. perimeter*), cirkularnost (eng. circularity), zaobljenost (*eng. roundness*), Feretov dijametar (*eng. Feret diameter*) i integrisana optička gustina (*eng. Integrated Optical Density).*

Statistička obrada podataka

Statistička analiza podataka rađena je u programskom paketu SPSS 15.0. Dobijeni rezultati su prikazani tabelarno.

Kontinualne varijable su predstavljene osnovnim statističkim parametrima – aritmetičkom sredinom ($\overline{X}$), standardnom devijacijom (SD), medijanom (Me) kao merom centralne tendencije, te opsegom, tj. minimalnim i maksimalnim vrednostima. Kvalitativna obeležja ispitivanih promenljivih data su učestalošću (n) i procentualnom zastupljenošću (%).

U zavisnosti od veličine uzorka, normalnost distribucije kontinualnih varijabli, ispitivana je Kolmogorov-Smirnov ili Shapiro-Wilkovim testom.

Za ocenu značajnosti razlike (p) kontinualnih varijabli između dve nezavisne grupe ispitanika korišćeni su Studentov t-test nezavisnih uzoraka, kod normalne distribucije podataka, ili Mann-Whitnijev U test, kod distribucije koja odstupa od normalne. Kao prag statističke značajnosti definisana je standardna vrednost, $p < 0,05$.

Za testiranje značajnosti razlike između više nezavisnih grupa korišćena je ANOVA, a na osnovu testiranja homogenosti varijansi po Levenu sprovedena je sledstvena Post Hoc analiza, odnosno multipna poređenja Tukey HSD (za homogene varijanse) ili Tamhaneovim testom u slučaju nehomogenosti varijansi.

Za testiranje statističke značajnosti razlika apsolutnih frekvencija između uzoraka korišćen je χ^2 test, ili Fisherov test egzaktne verovatnoće, ukoliko je apsolutna frekvencija obeležja manja od 5.

software, Wayne Rasband, National Institutes of Health, Bethesda, Maryland, USA). Photomicrographs, in colour, were obtained using a high-resolution digital camera (Nikon, DS-Fil, Tokyo, Japan), which was connected to the microscope (Nikon, ECLIPLE 50i, Tokyo, Japan). After that, the picture was transferred to the compatible computer and the analysis of nuclear parameters was carried out using the pack. The eight-bit picture was manually processed after the calibration, using a computer mouse. We analyzed 100 random tumor cell nuclei, at the magnification of x40, i.e. cells that did not overlap. We also analyzed six nuclear parameters: area, perimeter, circularity, roundness, Feret diam-eter and integrated optical density.

Statistical data analysis

The statistical analysis of data was performed in the program pack SPSS 15.0. The obtained results are presented in tables.

Continuous variables are presented by basic statistical parameters: mean value ($\overline{X}$), standard deviation (SD), median (Me) as a measure of central tendency, range, i.e. minimal and maximal values. Quantitative characteristics of the examined variables were determined based on frequency (n) and the percentage share (%).

Depending on the size of the sample, the normality of the distribution of continuous variables was examined using the Kolmogrov-Smirnov or Shapiro-Wilk test.

To evaluate the significance of the difference (p) of continuous variables between two independent groups of subjects, the Student's t-test of independent samples was used in case of the normal distribution of data, i.e. the Mann-Whitney U test in case of the distribution which deviates from the normal one. The standard value $p < 0.05$ was defined as the threshold of statistical significance.

ANOVA was used to test the significance of the difference between several independent groups, and based on the Levene's test for the homogeneity of variances, a sequential post-hock analysis was carried out, i.e. multiple comparisons using the Tukey's HSD (for homogenous variances) or the Tamhane's test in cases of non-homogenous variances.

To test the statistical significance of differences in absolute frequencies between samples, we used the χ^2 test, or the Fisher's exact probability test, if the absolute frequency of a sample was less than 5.

Rezultati

Na osnovu već iznetih mikromorfoloških karakteristika, analizirano je ukupno 44 tumora pljuvačnih žlezda, i to 20 pleomorfnih adenoma (Slika 1), 12 adenoma bazalnih ćelija (Slika 2) i 12 polimorfnih adenokarcinoma niskog gradusa (Slika 3 i 4).

Rezultati imunohistohemijske analize ekspresije markera proliferacije i intermedijarnih filamenata, kao i ostalih markera koji su analizirani u istraživanju prikazani su u Tabeli 2.

Ekspresija proteina se prati u duktalnoj i mioepitelnoj komponenti pleomorfnog adenoma. Neoplastične ćelije su pokazale jaku ekspresiju GFAP (20/20), p63 (20/20), WT1 (20/20), vimentin (18/20) i S100 (16/20) (Slika 5).

U grupi adenoma bazalnih ćelija analizirali smo sva četiri tipa rasta tumora. Ukupno je bilo dvanaest slučajeva iz ove grupe tumora. Najbrojniji je, generalno, solidni tip rasta. Praćena je ekspresija u luminalnim i bazaloidnim ćelijama. Odsustvo pozitivne reakcije primećeno je za GFAP. WT1, CEA i S100 su pokazali fokalnu pozitivnost, vimentin i CK14 su se eksprimirali u bazaloidnim ćelijama u tubularnom tipu, odnosno na periferiji tumorskih plaža u solidnom tipu rasta (Slika 6).

U grupi od dvanaest polimorfnih adenokarcinoma niskog gradusa prisutna je difuzna ekspresija CK14 (12/12). S100, vimentin i EMA su bili apsolutno eksprimirani, dok je αSMA bio negativan. Uočena je fokalna ekspresija WT1, naročito u kribriformnim delovima tumora (Slika 7).

Analizom vrednosti proliferativnog Ki67 indeksa ustanovljena je statistički značajna razlika u grupi pleomorfnog adenoma (Tumor mixtus), što se dovodi u vezu sa čestim recidiviranjem ovog benignog tumora.

Rezultati analize morfometrijskih karakteristika

Analizom morfometrijskih karakteristika uočavaju se veće vrednosti u grupi polimorfnog adenokarcinoma niskog gradusa, ali statistički značajne razlike nađene su samo za Feretov dijametar i integrisanu optičku gustinu ($p < 0,05$), kao i veće vrednosti za integrisanu optičku gustinu u grupi adenoma bazalnih ćelija ($p < 0,001$)

Results

Based on the already outlined micromorphological characteristics, we analyzed a total of 44 salivary gland tumors – 20 pleomorphic adenomas (*Figure 1*), 12 basal cell adenomas (*Figure 2*), and 12 polymorphous low-grade adenocarcinomas (*Figure 3 and 4*).

The results of immunohistochemical analysis of the expression of proliferation markers and intermediary filaments, as well as other markers analyzed in the study, are shown in Table 2.

The expression of proteins was monitored in the ductal and myoepithelial component of pleomorphic adenoma. Neoplastic cells showed a strong expression of GFAP (20/20), p63 (20/20), WT1 (20/20), vimentin (18/20) and S100 (16/20) (Figure 5).

We analyzed all four types of tumor growth in the basal cell adenoma group. Twelve cases from this tumor group were present. In general, the most common was the solid tumor growth. The expression in luminal and basal cells was also monitored. The absence of a positive reaction was noticed for GFAP. WTI, CEA, S100 showed focal positivity, vimentin and CK14 were expressed in basaloid cells in the tubular type, i.e. at the periphery of tumor nests in the solid growth type (Figure 6).

Diffuse expression of CK14 (12/12) was present in the group of 12 polymorphous low-grade adenocarcinomas. S100, vimentin and EMA were absolutely expressed, whereas αSMA was negative. Focal expression of WT1 was noticed, especially in the cribriform parts of the tumor (Figure 7).

The analysis of the proliferative Ki67 index values showed a statistically significant difference in the pleomorphic adenoma group (Tumor mixtus), which was associated with frequent recurrence of this benign tumor.

Results of the analysis of morphometric characteristics

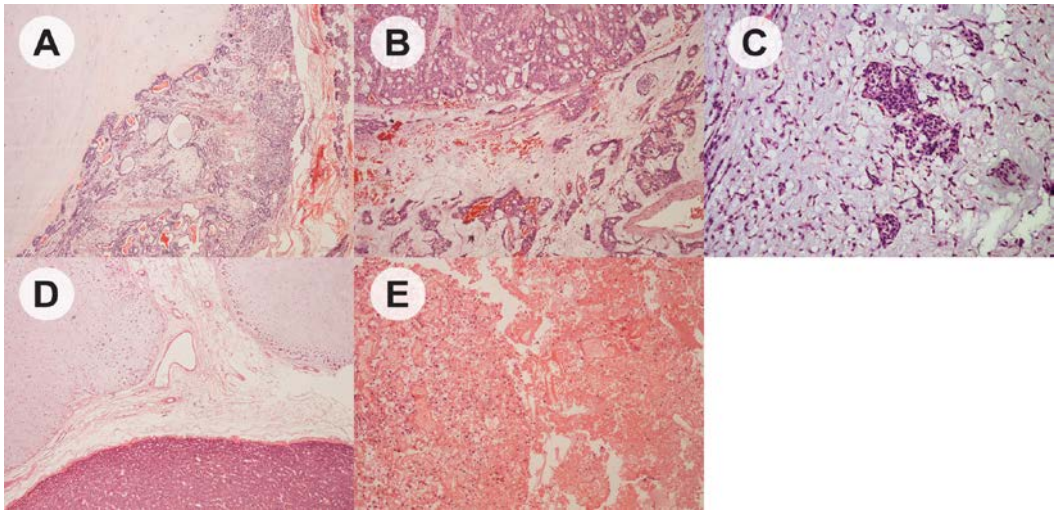
The analysis of morphometric characteristics showed higher values in the polymorphous low-grade adenocarcinoma group, but statistically significant differences were found only for Feret diameter and integrated optical density ($p < 0.05$), as well as higher values for integrated optical density in the basal cell adenoma group ($p < 0.001$).

Diskusija

Velika dijagnostička dilema može da nastane u diferencijaciji pleomorfnog adenoma i polimorfnog adenokarcinoma niskog gradusa. To je naročiti problem kada se govori o malim, incizionim biopsijama. U tom slučaju, može se stvoriti lažna slika o infiltrativnom rastu, ako je reč o tumorima malih pljuvačnih žlezda, gde pleomorfni adenom najčešće ne poseduje kapsulu, a može imati i fokalne ekstenzije u susedne žlezde i prezentovati se kao maligna neoplazma. Oba tipa tumora se sastoje od relativno uniformnih ćelija koje karakteriše odsustvo atipije [17]. Veoma bitan parametar o malignitetu, kada je reč o polimorfnom adeno-karcinomu niskog stepena, jeste perineuralna invazija, o kojoj se ne može izjašnjavati na malom biopsijskom uzorku.

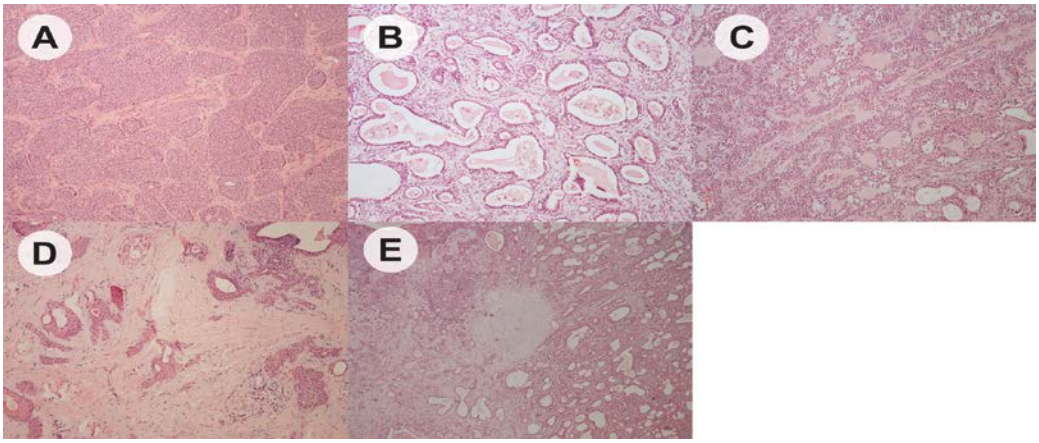
Discussion

A great diagnostic dilemma may arise in the differentiation of pleomorphic adenoma and polymorphous low-grade adenocarcinoma. It may be a problem especially when it comes to small, incisional biopsies. It that case, a false picture about the infiltrative growth may be created regarding minor salivary gland tumors where pleomorphic adenoma does not possess the capsule, but may have focal extensions in the neighboring glands and present itself as a malignant neoplasm. Both tumor types consist of relatively uniform cells which are characterized by the absence of atypia [17]. When it comes to polymorphous low-grade carcinoma, the perineural invasion is a very important parameter for malignity, and it cannot be determined based on a small biopsy sample.



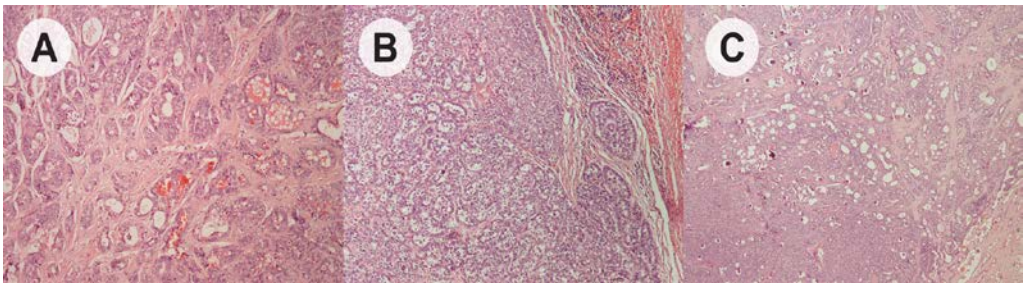
***Slika 1*. Pleomorfni adenom**, **A** – epitelna komponenta tumora formira solidno-cistične plaže sa hondroidnom stromom, H&E, uveličanje x4; **B** – celularni tip tumora sa tubularnom epitelnom komponentom, H&E, uveličanje x10; **C** – dominanta mukoidna stroma tumora, H&E, uveličanje x20; **D** – sinhroni rast pleomorfnog adenoma i mioepitelioma, H&E, uveličanje x4; **E** – područje ishemijske nekroze, H&E, uveličanje x10

***Figure 1*. Pleomorphic adenoma; A –** epithelial tumor component forms solid-cystic nests with chrondroid stroma, H&E, magnification x4; B – cellular tumor type with tubular epithelial component, H&E, magnification x10; **C** - dominant mucoid tumor stroma, H&E, magnification x20; **D** – synchronous growth of pleomorphic adenoma and myoepithelioma, H&E, magnification x4; **E** – area of ischemic necrosis, H&E, magnification x10;



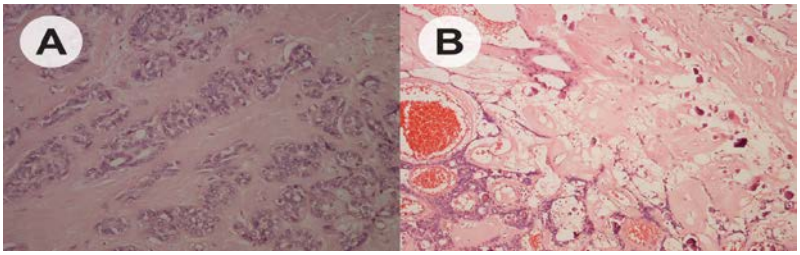
***Slika 2*. Adenom bazalnih ćelija**; **A** – solidni tip rasta tumora, H&E, uveličanje x10; **B** - tubularni tip rasta tumora, H&E, uveličanje x10; **C** - trabekularni tip rasta tumora, H&E, uveličanje x10; **D** - membranozni tip rasta tumora, H&E, uveličanje x10; **E** - tubularni tip rasta tumora sa područjem hondroidne metaplazije, H&E, uveličanje x10

***Figure 2*. Basal cell adenoma**; **A** – solid tumor growth type, H&E, magnification x10; **B** - tubular tumor growth type, H&E, magnification x10; **C** - trabecular tumor growth type, H&E, magnification x10; **D** - membranous tumor growth type, H&E, magnification x10; **E** - solid tubular growth type with area of chondroid metaplasia, H&E, magnification x10;



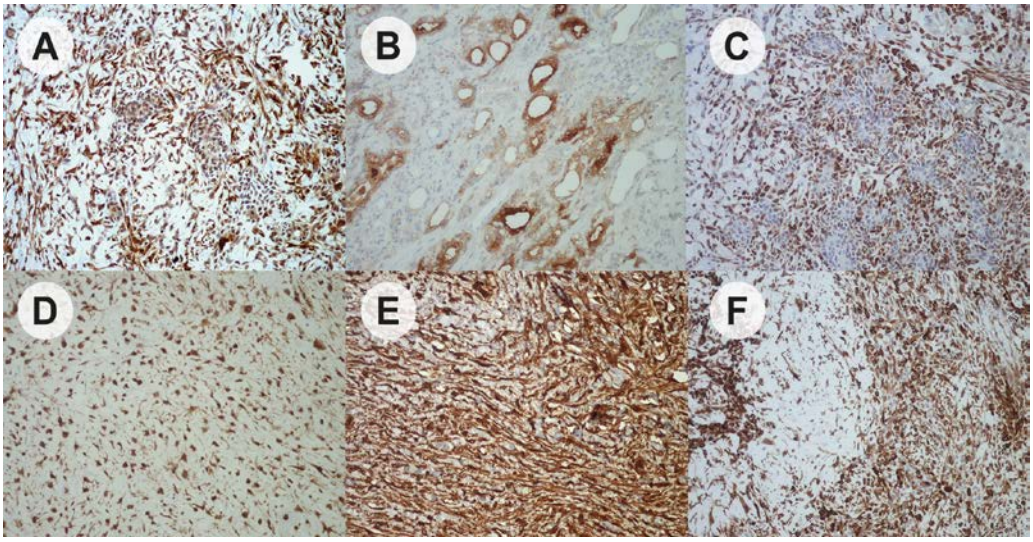
***Slika 3*. Polimorfni adenokarcinom niskog gradusa**, **A** – tubularni tip rasta, H&E, uveličanje x10; **B** – solidno-kribriformni tip rasta, H&E, uveličanje x10, **C** – solidno-cistični tip rasta, H&E, uveličanje x4

***Figure 3*. Polymorphous low-grade adenocarcinoma**; **A** – tubular growth type, H&E, magnification x10; **B** – solid-cribriform growth type, H&E, magnification x10, **C** – solid-cystic growth type, H&E, magnification x4;



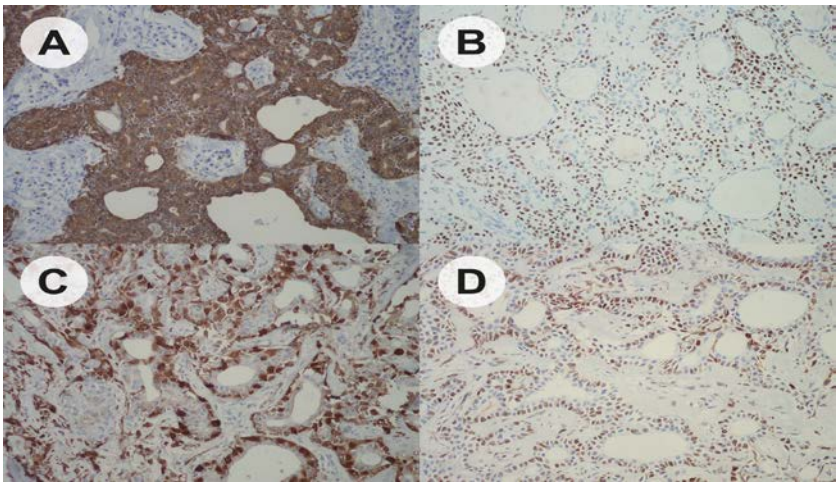
***Slika 4*. Polimorfni adenokarcinom niskog gradusa**, **A** – tubularni tip rasta tumora sa hijalinizovanom stromom, H&E, uveličanje x10; **B** – kribriformni tip rasta tumora sa mukoidnom stromom i mikrokalcifikatima, H&E, uveličanje x10

***Figure 4*. Polymorphous low-grade adenocarcinoma**; **A** – tubular growth type with hyalinized stroma, H&E, magnification x10; **B** – cribriform growth type with mucoid stroma and microcalcifications, H&E, magnification x10;



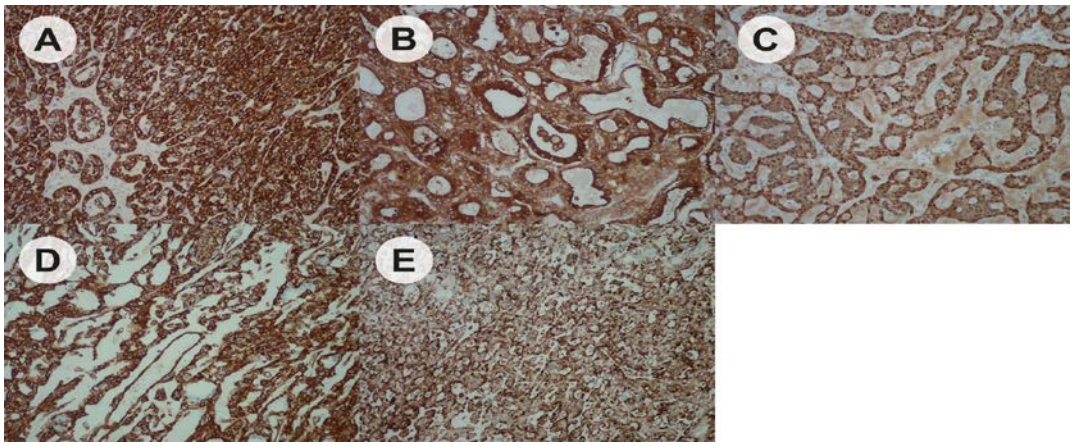
Slika 5. Tumor mixtus, uveličanje x20; **A** – Difuzna, intenzivna ekspresija αSMA u mioepitelnoj komponenti tumora; **B** – Ekspresija CEA u luminalnim ćelijama; **C** – intenzivna citoplazmatska ekspresija GFAP u epitelnim i mioepitelnim ćelijama; **D** – Jedarna ekspresija S100; **E** – Citoplazmatska ekspresija vimentina; **F** – Ekspresija WT1 u citoplazmi neoplastičnih mioepitelnih ćelija

Figure 5. **Tumor mixtus**; magnification x20; **A** – diffuse, intensive expression of αSMA in myopithelial tumor component; **B** – expression of CEA in luminal cells; **C** – intensive cytoplasmic expression of GFAP in epithelial and myoepithelial cells; **D** – nuclear expression of S100; **E** – cytoplasmic expression of vimentin; **F** – expression of WT1 in the cytoplasm of neoplastic myoepithelial cells;



Slika 6. Basal cell adenoma, uveličanje x20; **A** – CK14 – intenzivna citoplazmatska ekspresija u neoplastičnim ćelijama; **B** – Jedarna ekspresija p63 u bazaloidnim ćelijama; **C** – Jedarna i citoplazmatska ekspresija S100 u bazaloidnim ćelijama; **D** – Intenzivna citoplazmatska i slaba jedarna ekspresija WT1 u bazaloidnim ćelijama tumorskih plaža

Figure 6. **Basal cell adenoma**; magnification x20; **A** – CK14 – intensive cytoplasmic expression in neoplastic cells; **B** – nuclear expression of p63 in basaloid cells; **C** – nuclear and cytoplasmic expression of S100 in basaloid cells; **D** – intensive cytoplasmic and weak nuclear expression of WT1 in basaloid cell of tumor nests;



Slika 7. Polymorphus low-grade adenocarcinoma, uveličanje x20; **A** – Difuzna i intenzivna ekspresija CK14; **B** – Ekspresija EMA u luminalnim i abluminalnim ćelijama; **C** – Homogeno prebojavanje neoplastičnih ćelija S100; **D** – Citoplazmatska ekspresija vimentina; **E** – Ekspresija WT1 u kribriformnom delu tumora

Figure 7. **Polymorphous low-grade adenocarcinoma**, magnification x20; **A** – diffuse and intensive expression of CK14; **B** – expression of EMA in luminal and abluminal cells; **C** – homogenous S100 staining of neoplastic cells; **D** – cytoplasmic expression of vimentin; **E** – expression of WT1 in cribriform part of the tumor;

Tabela 1. Karakteristike pacijenata
Table 1. Characteristics of patients

Tumor type Tip tumora	Number (N) Broj (N)	Sex (man/woman) Pol (muškarac/žena)	Mean age Srednja starost
Pleomorphic adenoma Pleomorfni adenom	20	12/8	40
Basal cell adenoma Adenom bazalnih ćelija	12	4/8	67
Polymorphus low-grade adenocarcinoma Polimorfni adenokarcinom niskog gradusa	12	4/8	64

Tabela 2. Imunohistohemijska ekspresija
Table 2. Immunuhistochemical expression

Tumor type Tip tumora	CEA	EMA	Vimentin	p63	αSMA	S100	CK14	WT1	GFAP	p53	HE R2
Tumor mixtus Tumor mixtus	+ (10/20) L	+ (10/20) L	+ (18/20) ABL	+ (20/20) ABL	+ (14/20) ABL	+ (16/20) ABL	+ (14/20) L/ABL	+ (20/20) ABL	+ (20/20) ABL	+ (2/20) L	- (0/2 0)
Basal cell adenoma	+ (6/12) L	+ (8/12) L	+ (12/12) ABL	+ (12/12) ABL	+ (10/12) ABL	+ (8/12) ABL	+ (12/12) ABL	+ (6/12) ABL	- (0/12)	+ (6/12) L/ABL	- (0/1 2)
Polymorphus low-grade adenocarcinoma	+ (10/12) L/ABL	+ (12/12) L/ABL	+ (12/12) ABL	+ (2/12) ABL	- (0/12)	+ (12/12) L/ABL	+ (12/12) L/ABL	+ (4/12) ABL	- (0/12)	+ (6/12) L/ABL	- (0/1 2)

+ prisutna ekspresija proteina; - odsutna ekspresija proteina; ABL – abluminalne ćelije; L – luminalne ćelije
+ present protein expression; - absent protein expression; ABL – abluminal cells; L – luminal cells

Tabela 3. Vrednosti proliferativnog indeksa u odnosu na tip tumora
Table 3. Proliferative index values regarding the tumor type

Tumor type Tip tumora	X ± SD	(Me)	Min –	Max
Tumor mixtus	5,97 ± 4,05 *	(4,60)	1,20 –	13,00
Basal cell adenoma	3,85 ± 3,71	(2,34)	0,76 –	12,00
Polymorphus low grade adenocarcinoma	2,57 ± 1,32	(2,78)	1,18 –	4,78

Podaci su predstavljeni kao X ± SD (Me) Min–Max
* – p < 0,05
Data are presented as X ± SD (Me) Min–Max
* – p < 0,05

Tabela 4. Morfometrijske karakteristike jedara tumorskih ćelija
Table 4. Morphometric characteristics of tumor cell nuclei

		Basal cell adenoma (n = 12)	Polymorphus low-grade adenocarcinoma (n = 12)
Area	35,82 ± 3,57	40,97 ± 7,70	54,23 ± 12,38
Perimeter	23,28 ± 1,37	24,04 ± 2,09	27,85 ± 2,98
Circularity	0,83 ± 0,02	0,87 ± 0,02	0,86 ± 0,02
Feret diameter	8,66 ± 0,49	9,07 ± 0,65	10,42 ± 0,90*
IntDent	4,53 ± 0,38	15,81 ± 4,54 **	18,71 ± 6,79*
Roundness	0,69 ± 0,04	0,71 ± 0,05	0,70 ± 0,05

Podaci su predstavljeni kao X ± SD (Me) Min–Max
* – p < 0,05, ** – p < 0,001 (ANOVA, Post Hoc Test, Tamhane test)

Data are presented as X ± SD (Me) Min–Max
* – p < 0,05, ** – p < 0,001 (ANOVA, Post Hoc Test, Tamhane's test)

U ovakvim slučajevima neophodna je primena imunohistohemije. U prvom redu se primenjuju markeri mioepitelne diferencijacije i GFAP. Nakazato i saradnici su još 1982. godine ukazali na ukrštenu ekspresiju GFAP, vimentina i citokeratina u diferencijaciji pleomorfnog adenoma [18].GFAP pokazuje veoma izraženu citoplazmatsku ekspresiju u duktalnim epitelnim i mioepitelnim ćelijama adenoma, dok je zanemarljivo mala pozitivnost registrovana u ćelijama polimorfnog adenokarcinoma niskog gradusa [19]. Naši rezultati pokazuju potpuno odsustvo pozitivne reakcije GFAP kod ovog tipa karcinoma, a apsolutnu pozitivnost kod pleomorfnog adenoma, što je u skladu sa velikim studijama koje su se bavile diferencijalnom dijagnozom ova dva entiteta [20,21].

In such cases, it is necessary to apply immunohistochemistry. Firstly, markers of myoepithelial differentiation and GFAP are applied. Back in 1982, Nakazato et al. pointed to the cross-over expression of GFAP, vimentin and cytokeratin in the differentiation of pleomorphic adenoma [18] .GFAP exhibited a very complex cytoplasmic expression in ductal epithelial and myoepithelial adenoma cells, whereas insignificant positivity was registered in the cells of polymorphous low-grade adenoma [19]. Our results show a complete absence of the positive reaction of GFAP in this type of carcinoma, and absolute positivity in pleomorphic adenoma, which is an accordance with large studies dealing with the differential diagnosis of these two entities [20,21].

Takođe, veoma značajan nalaz u diferencijaciji jeste i ekspresija αSMA u pleomorfnom adenomu koja je odsutna u polimorfnom adenokarcinomu niskog gradusa [22]. Za dokazivanje neoplastičnih (modifikovanih) mioepitelnih ćelija, u poslednje vreme, koristi se WT1. Leader i Langman su u svojim studijama ukazivali na značaj ovog markera. WT1 je pokazao apsolutnu citoplazmatsku pozitivnost u abluminalnim ćelijama sa mioepitelnom diferencijacijom pleomorfnog adenoma [23]. Te ćelije su pokazale i ekspresiju p63. Kod polimorfnog adenokarcinoma niskog gradusa ekspresija je varirala u zavisnosti od varijante ćelijskog aranžmana. U skladu sa nalazima objavljenih studija, naši rezultati ukazuju na značaj imunohistohemijskog dokazivanja mioepitelnih ćelija u dijagnostici polimorfnog adenokarcinoma niskog gradusa.

Adenom bazalnih ćelija je uveden u klasifikaciju Svetske zdravstvene organizacije 1991. godine. Do tada je označavan kao nepleomorfni adenom ili monomorfni adenom. Na osnovu histološke prezentacije i načina rasta i rasporeda neoplastičnih ćelija, podeljen je u više varijanti, solidni, koji je i najčešći, tubularni, trabekularni i membranozni [15]. Uglavnom se u tumoru viđa više varijanti načina rasta, ali je jedna dominantna, na osnovu koje se dalje vrši morfološka subklasifikacija adenoma bazalnih ćelija. Svi imaju fibroznu stromu, ali bez miksohondroidnih područja, koja se viđaju u pleomorfnom adenomu. Cistična degeneracija, skvamozna metaplazija, keratinizacija i kribriformni rast su povećali mogućnost ovom tumoru za dijagnostičku grešku. Generalno, tumorske plaže su izgrađene od tamnih, plavih ćelija na periferiji, koje često imaju palisadni aranžman. U centralnim delovima su prisutne nešto krupnije ćelije, svetlije citoplazme. Kod svih varijanti postoji mioepitelna diferencijacija, koja na standardnim H&E preparatima nije očigledna, ali imunohistohemijskom analizom je dokazana [24]. U diferencijalnu dijagnozu adenoma bazalnih ćelija se uključuje polimorfni adenokarcinom niskog gradusa i pleomorfni adenom, kao i neke od varijanti mioepitelioma. Imunohistohemijskom analizom naših rezultata, a u skladu sa podacima iz literature, pokazali smo da ćelije na periferiji tumorskih plaža eksprimuju p63, vimentin, SMA i S100, dok su ćelije u unutrašnjosti ili luminalne, ako govorimo o tubularnoj varijanti, pozitivne na CK14, CEA i S100.

Moreover, a very significant finding in the differentiation includes the expression of αSMA in pleomorphic adenoma which is absent in polymorphous low-grade adenocarcinoma [22]. In recent years, WT1 has been used for the determination of neoplastic cells. Leader and Langman emphasized the significance of this marker in their studies. WT1 showed absolute cytoplasmic positivity in abluminal cells with myoepithelial differentiation of pleomorphic adenoma [23]. Those cells exhibited the expression of p69. In polymorphous low-grade adenocarcinoma, the expression varied depending on the variant of cell arrangement. In accordance with the findings of published studies, our results emphasize the importance of immunohistochemical detection of myoepithelial cells in the diagnostics of polymorphous low-grade adenocarcinoma.

Basal cell adenoma was classified by the World Health Organization in 1991. By that time, it had been referred to as non-pleomorphic adenoma or monomorphic adenoma. Based on the histological presentation, growth type and neoplastic cell arrangement, it was divided into several variants: solid, which is the most common, tubular, trabecular and membranous [15]. The tumor usually exhibits many growth type variants, with one of them being dominant, based on which further morphological subclassification of basal cell adenoma is done. They all have the fibrous stroma, but without myxochondroid areas which can be seen in pleomorphic adenoma. Cystic degeneration, squamous metaplasia, keratinization and the cribriform growth have increased the possibility of diagnostic errors for this tumor. In general, tumor nests consist of dark, blue cells at the periphery, which often have a palisade arrangement. Somewhat larger cells of lighter cytoplasm occupy the central parts. Myoepithelial differentiation exists in all variants and it is not obvious on standard H&E preparations, however, the immunohistochemical analysis has proven it [24]. The differential diagnosis of basal cell adenoma includes polymorphous low-grade adenocarcinoma and pleomorphic adenoma, as well as some of myoepithelioma variants. The immunohistochemical analysis of our results, in accordance with data from the literature, showed that cells at the periphery of tumor nests express p63, vimentin, SMA and S100, whereas cells in the interior or luminal cells, if we talk about the tubular variant, were positive to SK14, CEA and S100.

Analizirajući ekspresiju markera, dolazimo do zaključka da je adenom bazalnih ćelija histogenetski poreklom od ćelija interkalatnih kanala [25].

Evaluacija mitotskog indeksa, pre svega Ki67 proliferativnog indeksa, pokazala se kao veoma bitan parametar u diferencijalnoj dijagnostici, predviđanju biološkog ponašanja i agresivnosti u mnogim tumorima. Našim istraživanjem smo obuhvatili 32 tumora pljuvačnih žlezda, benignih i malignih, analizirali smo proliferacioni indeks i u korelaciji sa ostalim kliničko-patološkim karakteristikama dobili smo značajne rezultate u diferencijalnoj dijagnostici ovih neoplazmi.

Za potrebe našeg istraživanja koristili smo 5% kao graničnu vrednost za Ki67 proliferativni indeks, ali u dostupnoj literaturi postoje studije koje su se vodile višim vrednostima za agresivnost tumora, i to >10% [26,27]. U našem istraživanju je najveću vrednost pokazao celularni rekurentni pleomorfni adenenom mekog nepca.

Slično rezultatima našeg istraživanja, Shida i saradnici su dobili nešto veće vrednosti Ki67 proliferativnog indeksa u grupi adenoma bazalnih ćelija [28]. Sa rezultatima Horii i saradnika poklapaju se naši rezultati, koji ukazuju na najveću proliferativnu aktivnost pleomorfnog adenoma u grupi benignih tumora [29]. Upoređujući ekspresiju p53 i Ki67, Saghravanian i saradnici navode da je od velikog značaja u diferencijaciji polimorfnog adenokarcinoma niskog gradusa i adenoidnocističnog karcinoma. U njihovoj studiji je 24% jedara tumorskih ćelija pokazalo ekspresiju Ki67, dok je svega 3,88% u grupi polimorfnog adenokarcinoma niskog gradusa. Na osnovu velike varijabilnosti dobijenih rezultata u našem istraživanju, a i od strane drugih istraživača, po našem mišljenju, jedan je od dokaza da niska vrednost proliferativnog indeksa nije uvek strogo povezana sa tumorom niskog gradusa. rezultati našeg istraživanja pokazuju da vrednost Ki67 proliferativnog indeksa varira u zavisnosti od slučaja do slučaja, kao i od histološkog tipa samog tumora.

Ispitivanje ekspresije p53 i Ki67 je široko zastupljena metoda u utvrđivanju evolucije i prognoze malignih tumora svih lokalizacija. Najčešće identifikovani mutirani gen u malignim neoplazmama je p53, naročito je zastupljen kod karcinoma dojke, želuca, jetre i prostate. Ekspresija p53 je udružena sa negativnom prognozom bolesti, i obično je prisutna kod agresivnih malignih tumora visokog gradusa. Mutacija gena je uočena i kod tumora pljuvačnih žlezda, ali su rezultati još uvek nedovoljni i nepotpuni.

Having analyzed the expression of markers, we concluded that basal cell adenoma was histogenetically of intercalated channel cells origin [25].

The evaluation of the mitotic index, primarily the Ki67 proliferative index, proved to be a very important parameter in the differential diagnosis, predicting biological behavior and aggressiveness in many tumors. Our study included 42 salivary gland tumors, both benign and malignant. We analyzed the proliferative index and, in correlation with other clinical-pathological characteristics, we obtained significant results in the differential diagnosis of these neoplasms.

For the purposes of our study, we used 5% as a threshold for the Ki67 proliferative index, but the existing literature offers studies which were conducted using higher values for tumor aggressiveness, i.e. >10% [26,27]. In our study, recurrent cellular pleomorphic adenoma of the soft palate showed the highest value.

Similar to the results of our study, Shida et al. obtained slightly higher values of the Ki67 proliferative index in the basal cell adenoma group [28]. Our results match the results of Horii et al., which indicate the highest proliferative activity of pleomorphic adenoma in the group of benign tumors [29]. By comparing the expressions of p53 and Ki67, Saghravanian et at. state that they are of great importance for the differentiation of polymorphous low-grade adenocarcinoma and adenoid cystic carcinoma. In their study, 24% of tumor cell nuclei showed the expression of Ki67, contrary to only 3.88% in the polymorphous low-grade adenocarcinoma group. Based on great variability of the obtained results in our study, as well as results from other researchers, we believe that a low value of the proliferative index is not necessarily always strictly related to a low-grade tumor. The results of our study show that the value of the Ki67 proliferative index varies depending on the case and the histological type of the tumor itself.

The examination of the expression of p53 and Ki67 is a widespread method in the determination of the evolution and prognosis of malignant tumors of all localizations. p63 is the most commonly identified mutated gene in malignant neoplasms, especially present in breast, stomach, liver and prostate cancer. The expression of p53 is associated with the negative prognosis of a disease, and it is usually present in aggressive malignant

Weber je sa svojim saradnicima analizirao ekspresiju p53 u grupi benignih tumora. Pokazali su da ekspresija proteina postoji jedino u grupi pleomorfnog adenoma i mioepitelioma. Svoje rezultate su tumačili kao povećanu sklonost tumora ka malignoj alteraciji [30].

Korišćenjem softverskog paketa „ImageJ" analizirano je šest jedarnih parametara: površina, perimetar, cirkularnost, zaobljenost, Feretov dijametar i integrisana optička gustina.

Razlike nađene za jedarne parametre, koji se odnose na veličinu jedara (površina i perimetar), kao i na oblik jedra (cirkularnost i zaobljenost), minimalno su bile veće u grupi polimorfnog adenokarcinoma niskog gradusa. Vrednosti za Feretov dijametar i integrisane optičke gustine statistički značajno zavise od prirode i tipa tumora ($p<0,05$).

U nama dostupnoj literaturi, generalno, jako je malo studija koje su se bavile morfometrijskim karakteristikama tumora. Već svetlosnom mikroskopijom možemo da uočimo neke od karakteristika jedara. Jasno se vidi da li je reč o hiperhromnim jedrima, krupnim ili malim, da li je prisutna atipija, kakav je hromatin, da li su ivice ravne, površina nazupčana. Diferencijalna dijagnoza tumora pljuvačnih žlezda je često veoma teška, a morfometrija omogućava kvantifikaciju patohistološkog nalaza, a samim tim i smanjuje mogućnost greške u postavljanju definitivne dijagnoze. Morfometrijska analiza tumora pljuvačnih žlezda je do sada primenjivana i mali je broj studija koje su komparirale rezultate ovih analiza u različitim tipovima tumora. U dosadašnjim istraživanjima autori su uglavnom istraživali jedarnu površinu, cirkularnost, perimetar i integrisanu optičku gustinu, korišćenjem različitih softverskih paketa [31,32]. Layfield je u svojoj studiji objavio rezultate vezane za ove parametre i pokazao njihovu dijagnostičku značajnost u diferencijaciji benignih i malignih mešovitih tumora parotidne žlezde [33]. Prvulović je sa svojim saradnicima objavila studiju u diferencijaciji duktalnog i lobularnog karcinoma dojke na citološkom materijalu. Takođe je istakla i primenjivost ove metode, ne samo u diferencijaciji, već i u gradiranju samog karcinoma [34]. Oz i saradnici su objavili studiju koja se bavila morfometrijskim karakteristikama ćelija oralne sluzokože kod bolesnika sa dijabetesom

high-grade tumors. Gene mutation has also been noticed in salivary gland tumors, but the results are still insufficient and incomplete.

Weber et al. analyzed the expression of p53 in the benign tumor group.
They showed that the expression of protein existed only in the pleomorphic adenoma and myoepithelioma groups. They interpreted their results as an increased tendency of tumors to malignant alteration [30].

Six nuclear parameters were analyzed using the software pack "ImageJ": area, perimeter, circularity, roundness, Feret diameter and integrated optical density.

The differences found for the nuclear parameters which refer to the size of nuclei (area and perimeter), as well as the shape of nuclei (circularity and roundness), were slightly higher in the polymorphous low-grade adenocarcinoma group. The values of Feret diameter and integrated optical density significantly depend on the nature and type of the tumor ($p < 0.05$).

The available literature offers a very small number of studies dealing with the morphometric characteristics of tumors. Using light microscopy, we can notice some of the characteristics of nuclei. It can be clearly seen whether it is the case of hyperchromatic nuclei, large or small, whether atrophy is present, what chromatin is like, whether the edges are even, or the area serrated. The differential diagnosis of salivary gland tumors is often quite difficult, and the morphometry enables the quantification of a pathohistological finding, and therefore reduces the possibility for errors in setting the final diagnosis. The morphometric analysis of salivary gland tumors has been applied so far, but the number of studies which have compared the results of these analyses in different tumor types is rather small. In previous studies, the authors mostly examined nuclear area, circularity, perimeter and integrated optical density using different software packs [31,32]. Layfield published the results for these parameters in his study, and showed their diagnostic significance in the differentiation of benign and malignant mixed tumors of the parotid gland [33]. Prvulović et al. published a study on the differentiation of ductal and lobular breast carcinoma on cytological material. They also emphasized the applicability of this method, not only in the differentiation, but also in grading the ca-

melitusom tip I, i pokazali kako su jedarni parametri statistički veći u ovoj grupi bolesnika [35]. Obad-Kovačević je u svoje istraživanje uključila morfometrijske karakteristike benignih i malignih tumora parotidne žlezde, proučavajući karakteristike cele ćelije. Došli su do zaključka da je odnos površine jedara i citoplazme znatno veći, u korist jedara, kod malignih tumora [36].

Zaključak

Plemorfni adenomi su pozitivni na S-100, GFAP, CK14, αSMA, CEA, EMA i WT1. Adenom bazalnih ćelija pokazuje pozitivnost na S-100, CEA, p63 i vimentin. Ekspresija HER2 u grupi benignih tumora bila je u potpunosti odsutna. Polimorfni adenokarcinom niskog gradusa pokazao je pozitivnu imunohistohemijsku reakciju na CK14, p63, EMA, S100 i vimentin.

Najveću proliferativnu aktivnost pokazuje pleomorfni adenom. Dobijeni rezultati su u skladu sa frekvencijom recidi-viranja tumora, kao i sa stepenom maligne alteracije.

Vrednosti morfometrijskih parametara, integrisana optička gustina i Feretov dijametar statistički su veće u grupi polimorfnog adenokarcinoma niskog gradusa. U grupi adenoma bazalnih ćelija vrednosti za integrisanu optičku gustinu statistički su veće u odnosu na grupu pleomorfnih adenoma.

rcinoma itself [34]. Oz et al. published a study on the morphometric characteristics of oral mucosa cells in patients with diabetes mellitus type I, and showed that nuclear parameters were higher in this group of patients [35]. Obad-Kovačević et al. included morphometric characteristics of benign and malignant tumors of the parotid gland in their research by studying the characteristics of the entire cell. They concluded that the ratio of the area of nuclei and cytoplasm was considerably higher in malignant tumors, in favor of nuclei[36].

Conclusion

Pleomorphic adenoma was positive to S-100, GFAP, CK14, αSMA, CEA, EMA and WT1, whereas basal cell adenomas showed positivity to S-100, CEA, p63 and vimentin. The expression of HER2 in the benign tumor group was completely absent. Polymorphous low-grade adenocarcinoma showed a positive immunohistochemical reaction to CK14, p63, EMA, S100 and vimentin.

Pleomorphic adenoma showed the highest proliferative activity. The obtained results were in accordance with the frequency of tumor recurrence, as well as the degree of malignant alteration.

The values of morphometric parameters – integrated optical density and Feret diameter – were statistically higher in the polymorphous low-grade adenocarcinoma group. The basal cell adenoma group showed statistically higher values for integrated optical density compared to the pleomorphic adenoma group.

.

LITERATURA / REFERENCES

1. de Oliveira FA, Duarte EC, Taveira CT, Máximo AA, de Aquino EC, Alencar Rde C, et al. Salivary gland tumor: a review of 599 cases in a Brazilian population. Head Neck Pathol. 2009; 3(4):271-275.
2. Guzzo M, Locati LD, Prott FJ, Gatta G, McGurk M, Licitra L. Major and minor salivary gland tumors. Crit Rev Oncol Hematol. 2010; 74(2):134-138.
3. Ettl T, Schwarz-Furlan S, Gosau M, Reichert TE. Salivary gland carcinomas. Oral Maxillofac Surg. 2012; 16:267-283.
4. Lukšić I, Virag M, Manojlović S, Macan D. Salivary gland tumours: 25 years of experience from a single institution in Croatia. J Craniomaxillofac Surg. 2012; 40(3):e75-81.
5. Fonseca FP, Carvalho Mde V, de Almeida OP, Rangel AL, Takizawa MC, Bueno AG, et al. Clinicopathologic analysis of 493 cases of salivary gland tumors in a Southern Brazilian population. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012; 114(2):230-239.
6. Vasconcelos AC, Nör F, Meurer L, Salvadori G, Souza LB, Vargas PA, et al. Clinicopathological analysis of salivary gland tumors over a 15-year period. Braz Oral Res. 2016; 30:e2.
7. Barnes L, Eveson JW, Reichart P, Sidransky D. World Health Organization classification of tumours. Pathology and genetics of head and neck tumours. Lyon: IARC, 2005.
8. Zhan KY, Khaja SF, Flack AB, Day TA. Benign Parotid Tumors. Otolaryngol Clin North Am. 2016; 49(2):327-342.
9. Khetrapal S, Jetley S, Hassan MJ, Jairajpuri Z. Cystic change in pleomorphic adenoma: a rare finding and a diagnostic dilemma. J Clin Diagn Res. 2015; 9(11): ED07-8.
10. Takamine K, Ueda Y, Nakano K, Ochiai T, Sugita Y, Kubo K, et al. Notch as a Possible Cell Differentiation Factor in Pleomorphic Adenomas. Int J Med Sci. 2015; 12(10):759-763.
11. Siefert G, Sobin LH. Histological classification of salivary gland tumours. In: World Health Organization, International histological classification of tumours. Berlin Springer, 1991.
12. Kanaujia SK, Singh A, Nautiyal S, Ashutosh K. Basal cell adenoma of parotid gland: case report and review of literature. Indian J Otolaryngol Head Neck Surg. 2015; 67(4):430-433.
13. Mayers EN, Ferris RL. Salivary Gland Disorders. Berlin, Heidelberg: Springer-Verlag, 2007.
14. González-García R, Nam-Cha SH, Muñoz-Guerra MF, Gamallo-Amat C. Basal cell adenoma of the parotid gland. Case report and review of the literature. Med Oral Patol Oral Cir Bucal. 2006; 11(2): E206-9.
15. Zarbo RJ. Salivary gland neoplasia: a review for the practicing pathologist. Mod Pathol. 2002; 15:298–323.
16. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med. 2010; 134:e48-72.
17. Ellis GL, Auclair PL. Tumors of the salivary glands. Atlas of Tumor Pathology, third series, Fascicle 11. Washington, DC: Armed Forces Institute of Pathology, 1996.
18. Nakazato Y, Ishizeki J, Takahashi K, Yamaguchi H, Kamei T, Mori T. Localization of S-100 protein and glial fibrillary acidic protein-related antigen in pleomorphic adenoma of the salivary glands. Lab Invest. 1982; 46(6):621-626.
19. Gnepp DR, el-Mofty S. Polymorphous low grade adenocarcinoma: glial fibrillary acidic protein staining in the differential diagnosis with cellular mixed tumors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1997; 83(6):691–695.
20. Castle JT, Thompson LD, Frommett RA, Wenig BM, Kessler HP. Polymorphous low-grade adenocarcinoma: a clinicopathologic study of 164 cases. Cancer. 1999; 86:207-219.
21. Curran AE, White DK, Damm DD, Murrah VA. Polymorphous low-grade adenocarcinoma versus pleomorphic adenoma of minor salivary glands: resolution of a diagnostic dilemma by immunohistochemical analysis with glial fibrillary acidic protein. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001; 91(2):194–199.
22. Prasad ML, Barbacioru CC, Rawal YB, Husein O, Wen P. Hierarchical cluster analysis of myoepithelial/basal cell markers in adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma. Mod Pathol. 2008; 21(2):105–114.
23. Langman G, Andrews CL, Weissferdt A. WT1 expression in salivary gland pleomorphic adenomas: a reliable marker of the neoplastic myoepithelium. Mod Pathol. 2011; 24:168–174.
24. Dardick I, Daley T, van Nostrand AW. Basal cell adenoma with myoepithelial cell-derived “stroma”: a new major salivary gland tumor entity. Head Neck Surg. 1986; 8:257–267.
25. de Sousa SOM, de Araújo NS, Corrêa L, Soubhia AMP, de Araújo VC. Immunohistochemical aspects of basal cell adenoma and canalicular adenoma of salivary glands. Oral Oncol. 2001; 37(4):365-368.
26. Norberg-Spaak L, Dardick I, Leidin T. Adenoid cystic carcinoma: use of cell proliferation, BCL-2 expression, histologic grade, and clinical stage as predictors of clinical outcome. Head Neck. 2000; 22(5):489–497.
27. Hellquist HB, Sundelin K, Di Bacco A, Tytor M, Manzotti M, Viale G. Tumour growth fraction and apoptosis in salivary gland acinic cell carcinomas. Prognostic implications of Ki-67 and bcl-2 expression and of in situ end labelling (TUNEL). J Pathol. 1997; 181(3):323–329.
28. Shida H, Tanaka A, Fukuda M, Shigematsu H, Kusama K, Sakashita H. A case of basal cell adenoma in the parotid gland. Jpn J Oral Maxillofac Surg. 2005; 51(7):352–355.

29. Horii A, Yoshida J, Sakai M, Okamoto S, Kubo T. Flow cytometric analysis of DNA content and Ki-67-positive fraction in the diagnosis of salivary gland tumors. Eur Arch Otorhinolaryngol. 1998, 255(5):265–268.
30. Weber A, Langhanki L, Schütz A, Gerstner A, Bootz F, Wittekind C, et al. Expression profiles of p53, p63, and p73 in benign salivary gland tumors. Virchows Arch. 2002; 441(5):428–436.
31. de Vicente Rodriguez JC, López Arranz JS, Vega Alvarez JA, Santos Oller JM, Gener González M. Morphometric study of epithelial neoplasms of the parotid gland. ev ADM. 1990; 47(4):215-219.
32. Gentile R, Zeppa P, Zabatta A, Vetrani A. The role of morphometry in the cytology of pleomorphic adenomas of the salivary glands. Pathologica. 1994; 86(2):167-169.
33. Layfield LJ, Hall TL, Fu YS. Discrimination of benign versus malignant mixed tumors of the salivary gland using digital image analysis. Cytometry. 1989; 10:217–221.
34. Prvulović I, Kardum-Skelin I, Sustercić D, Jakić-Razumović J, Manojlović S.Morphometry of tumor cells in different grades and types of breast cancer. Coll Antropol. 2010; 34(1):99-103.
35. Oz ZS, Bektas S, Battal F, Atmaca H, Ermis B. Nuclear morphometric and morphological analysis of exfoliated buccal and tongue dorsum cells in type-1 diabetic patients. J Cytol. 2014; 31(3):139-143.
36. Obad-Kovačević D, Kardum-Skelin I, Jelić-Puškarić B, Vidjak V, Blašković D. Parotid gland tumors: correlation between routine cytology and cytomorphometry by digital image analysis using conventional and newly introduced cytomorphometric parameters. Diagn Cytopathol. 2013; 41(9):776-784.

Primljen/ Recived on : 31.03.2017.
Revidiran/ Revised on : 18.04.2017.
Prihvaćen/ Accepted on : 28.04.2017.

ORGINALNI RAD
KLINIČKA STUDIJA
ORIGINAL ARTICLE
CLINICAL STUDY
doi:10.5937/asn1775696G

ANTIMIKROBNA SVOJSTVA AKRILATNIH SMOLA ZA STOMATOLOŠKE PROTEZE IMPREGNIRANIH NANOČESTICAMA SREBRA

ANTIMICROBIAL PROPERTIES OF ACRYLIC RESINS FOR DENTURES IMPREGNATED WITH SILVER NANOPARTICLES

Nikola Gligorijević [1], Milena Kostić [1], Ana Tačić [2], Ljubiša Nikolić [2], Vesna Nikolić [2]

[1]ODELJENJE STOMATOLOŠKE PROTETIKE, MEDICINSKI FAKULTET UNIVERZITET U NIŠU, SRBIJA
[2]TEHNOLOŠKI FAKULTET U LESKOVCU UNIVERZITETA U NIŠU, SRBIJA

[1]DEPARTMENT OF PROSTHETIC DENTISTRY, FACULTY OF MEDICINE, UNIVERSITY OF NIŠ, SERBIA
[2]UNIVERSITY OF NIŠ, FACULTY OF TECHNOLOGY IN LESKOVAC, LESKOVAC, SERBIA

Sažetak

Uvod. *Poroznost i površinska adherentnost akrilata čine ih kolektorima infektivnog sadržaja iz usne duplje. To se posebno odnosi na hladno polimerizujuće akrilate, čija je struktura manje kompaktna.*
Cilj *rada bio je ispitivanje antimikrobnog dejstva hladno polimerizujućeg akrilata nakon njegove impregnacije nanočesticama srebra.*
Materijal i metode. *Polimernoj komponenti (prahu) hladno polimerizovanog akrilata dodate su različite koncentracije (2%, 5% i 10%) nanočestica srebra, nakon čega su napravljeni uzorci oblika diska promera 10 mm. Kao kontrola poslužio je disk od nanočestica srebra. Antimikrobna aktivnost ispitivana je disk difuzionom metodom na dva česta izazivača infekcija usne duplje – Gram pozitivnoj bakteriji, Staphylococcus aureus ATCC 25923, i gljivici, Candidi albicans ATCC2091.*
Rezultati *su pokazali da uzorak čistog srebra, kao i uzorci polimera sa srebrom, pokazuju antibakterijsku aktivnost. Zona inhibicije rasta Staphylococcus aureusa na hranjivoj podlozi upravno je srazmerna koncentraciji nanočestica srebra u akrilatu. Sa druge strane, ispitivani uzorci nisu ihhibirali rast Candide albicans na hranjivoj podlozi.*
Zaključak. *Nanočestice srebra u akrilatu pokazale su antibakterijsku aktivnost. Proširenje njihovog spektra delovanja, kao i mogućnost eventualne kliničke primene biće predmet budućih istraživanja.*

Ključne riječi: nanočestice srebra, akrilati, antimikrobna aktivnost

Abstract

Introduction. *Porosity and surface adherence of acrylates make them collectors of infectious content from the mouth. This applies particularly to cold-curable acrylates, whose structure is less compact.*
The aim *of this study was to investigate the antimicrobial effects of cold polymerized dental acrylics after impregnation with silver nanoparticles.*
Material and Methods. *Different concentrations (2%, 5% and 10%) of silver nanoparticles were added to the polymer components (powder) of cold polymerizing acrylate, after which disk-shaped samples, 10 mm, in diameter, were made. A disk of silver nanoparticles was used as a control for our study. Antimicrobial activity was investigated using the disc diffusion method on the two most common oral cavitiy infecting agents - Gram positive bacterium, Staphylococcus aureus ATCC 25923, and fungus Candida albicans ATCC 2091.*
The results *have shown that the control sample as well as the samples of silver impregnated acrylics resins exhibit antibacterial activity. The growth zone inhibition of Staphylococcus aureus in the culture medium is proportional to the concentration of silver nanoparticles in the cold curing acrylic resin. On the other hand, the test samples did not inhibit the growth of Candida albicans in the medium.*
Conclusion. *Silver nanoparticles in cold curing acrylic resin demonstrated antibacterial activity. Expanding their antimicrobial spectrum of activity and their potential clinical application will be the subject of the future research.*

Key words: silver nanoparticles, acrylates, antimicrobial activity

Corresponding author:
Nikola Gligorijević, DDS,Phd Student
Department of Prosthetic Dentistry, Clinic of Dentistry,
Blvd. dr Z. Djindjića 52, 18000 Niš, Serbia
Tel. +381653366646;
E-mail:dr.gligorijevicnikola@gmail.com

Uvod

Usna duplja predstavlja stanište za široki spektar mikroorganizama, u koje spadaju bakterije, kvasci i virusi, gde se sve od navedenih grupa povezuju sa nastankom oralnih infekcija. Bakterije su najdominantnija komponenta mikroflore koja kolonizuje površinu zuba, sluzokožu i jezik, formirajući oralni biofilm [1,2].

Većina bakterijskih infekcija unutar usne duplje su polimikrobne i izuzetno je retko da se pronađe infekcija čiji je uzročnik samo jedna bakterijska vrsta. Pojedinačni udeo svake od bakterijskih vrsta prisutnih u takvim infekcijama je teško odrediti.

Bolesti vezane za dentalni plak su verovatno najčešće bakterijske bolesti koje se javljaju u usnoj duplji čoveka. Zubni karijes je stanje u kome dolazi do destrukcije tvrdih zubnih tkiva koja, ako se ne leči, može napredovati do zapaljenja i smrti tkiva zubne pulpe, sa mogućim širenjem infekcije u periapikalno područje zuba. U sam tok bolesti su uključene acidogene bakterije plaka - Streptococcus mutans, Streptococcus sobrinus i Lakto-Bacillus spp. [1].

Upotreba protetskih nadoknada menja uslove u usnoj duplji i promoviše deponovanje biofilma na dentalnim površinama protetskih nadoknada [3]. Proteze se prave od polimetil metakrilat (PMMA) akrilne smole [4]. Porozna površina i strukturne nepravilnosti akrilnih smola pogoduju akumulaciji mikroorganizama koji utiču na nastanak najvećeg broja bolesti usne duplje, kao što su kandidijaza, karijes, gingivitis i parodontopatije [5,6].

Saznanja o antimikrobnim svojstvima srebra (Ag) datiraju još od pre 3000 godina. Mehanizam njegovog antimikrobnog dejstva se zasniva na interakciji srebra sa tiol grupama enzima uključenim u ćelijski metabolizam bakterije, čime izaziva ćelijsku smrt [7].

Srebrne nanočestice mogu da ubiju sve patogene mikroorganizme, a još uvek nije zabeleženo da neki mikroorganizam ima sposobnost da razvije rezistentnost na njihovo delovanje. Nanočestice srebra su netoksične za ljude i veoma efikasne u borbi protiv bakterija, virusa i drugih eukariotskih mikroorganizama u veoma niskim koncentracijama i bez nuspojava [8]. Zbog ovih svojstava, joni srebra i srebrne nanočestice (AgNPs) se dodaju stomatološkim materijalima [9,10].

Cilj ovog istraživanja bio je da se procene antimikrobna svojstva nanostrukturnog srebra (576832 *Nanoprah koloidnog*

Introduction

The oral cavity provides habitats for a wide diversity of microorganisms, including bacteria, yeasts and viruses, with members of all groups being associated with oral infections. Bacteria are the predominant components of this resident microflora that colonizes surfaces on the teeth, mucosa and tongue, forming an oral biofilm [1,2].

Most bacterial infections within the oral cavity are polymicrobial in nature, and it is quite unusual to find any that is clearly due to a single species. The relative contribution of different bacterial components in such infections is thus difficult to determine.

Plaque-related diseases are probably the most common bacterial diseases occurring in men. Dental caries (dental decay) is a destructive condition of the dental hard tissues that, if uncontrolled, can progress to inflammation and death of the vital pulp tissue, with eventual spread of the infection to the periapical area of the tooth and beyond. The disease process involves acidogenic plaque bacteria, including Streptococcus mutans, Streptococcus sobrinus and Lactobacillus spp. [1].

The use of prosthetic devices within the oral cavity changes the oral conditions and promotes the deposit of biofilms on dental surfaces and on the prosthetic device [3]. Dentures are made of poly(methyl methacrylate) (PMMA) acrylic resin [4].The porous surface and irregularities of acrylic resins favor the accumulation of microorganisms, which are determining agents in the vast majority of oral problems, such as candidiasis, caries, gingivitis, and periodontitis [5,6].

The antimicrobial properties of silver (Ag) dates to 3000 years ago, and the mechanism is based on the interaction of silver with thiol groups of enzymes involved in bacterial cell metabolism thus causing cell death [7].

Silver nanoparticles can kill all pathogenic microorganisms, and no report as yet has shown that any organism can readily build up resistance to them. Silver nanoparticles are also reported to be nontoxic to humans and very effective against bacteria, viruses, and other eukaryotic micro-organisms at very low concentrations and without side effects[8].Due to this property, silver ions and silver nanoparticles (AgNPs) have also been introduced in dental materials [9,10].

srebra, Sigma-Aldrich) impregniranog u stomatološke akrilne smole za izradu zubnih proteza (poli (metil metakrilat), PMMA) u odnosu na *Candidu albicans i Streptococcus aureus,* najčešće patogene mikroorganizme koji se javljaju kod osoba koje nose zubne proteze.

Materijal i metode

Nanočestice srebra (*576832 Nanoprah koloidnog srebra, Sigma-Aldrich*) u koncentracijama od 2%, 5% i 10% dodati su praškastoj komponenti hladnopolimerizujuće akrilne smole (*Triplex Cold, Ivoclar Vivadent*). Uzorci su izrađeni prema uputstvima proizvođača i izliveni u kalupe oblika diska prečnika 10 mm. Dobijeni uzorci su zatim usitnjeni u prah za dalju upotrebu u eksperimentu. U našoj studiji je kao kontrola korišćen disk nanočestica srebra prečnika 10 mm.

Antimikrobna aktivnost ispitivana je korišćenjem disk difuzione metode [11]. Antimikrobna aktivnost sva četiri uzorka je testirana na Gram pozitivnu bakteriju - Staphilococcus aureus ATCC 25923 i gljivicu Candidu albicans ATCC 2091.

Podloge korišćene za rast mikroorganizama su hranljive agarne podloge za bakterije i Sabouraud maltozne agar podloge (Torlak, Beograd) za gljivice. Podloge su sterilisane 15 minuta u autoklavu na 121° C pod pritiskom od 110 kPa. U 10 cm^3 supstrata dodate su 0,1cm^3 adekvatne mikrobne kulture, a zatim presipane u petri šolje. Uzorci su postavljeni na inokulisanu površinu supstrata. Inkubacija je izvedena za 24 časa na 37° C za bakterije i 48 sati na 25° C za gljivice. Posle inkubacije, merene su zone inhibicije rasta i vrednosti su izražene u mm. Prisustvo zona inhibicije ukazuje na antimikrobnu aktivnost uzoraka.

Rezultati

Rezultati su pokazali da kontrolni uzorak (*Uzorak 23*), kao i uzorci nanosrebrom impregniranih akrila smola (*Uzorci 20-22*) ispoljavaju antibakterijsku aktivnost. Zona inhibicije rasta Staphilococcus aureus (*Slika 1*) u medijumu kulture je proporcionalna koncentraciji nanočestica srebra u hladnopolimerizovanoj akrilnoj smoli (*Tabela 1*). Test uzorci nisu inhibirali rast Candide albicans u medijumu (*Slika 2*).

The aim of this study was to evaluate the antimicrobial properties of nanostructured silver (*576832 Colloidal silver Nano powder, Sigma-Aldrich*) impregnated dental acrylic resins (poly(methyl methacrylate), PMMA) against *Candida albicans* and *Streptococcus aureus,* the main microorganisms associated with dental prostheses.

Material and Methods

Silver nanoparticles (576832 Colloidal silver Nano powder, Sigma-Aldrich) of 2%, 5% and 10%concentrations were added to the powder component of the cold curing acrylic resin (Triplex Cold, Ivoclar Vivadent). Samples were made according to the manufacturer's instructions and poured into a disk shapemold 10mmin diameter. The obtained samples were then shredded into powder for further usage in the experiment. A disk of silver nanoparticles was used as a control for our study.

Antimicrobial activity was investigated using the disc diffusion method[11]. Antimicrobial activity of all four samples was tested on Gram positive bacterium-Staphylococcus aureus ATCC 25923 and fungus Candida albicans ATCC 2091.

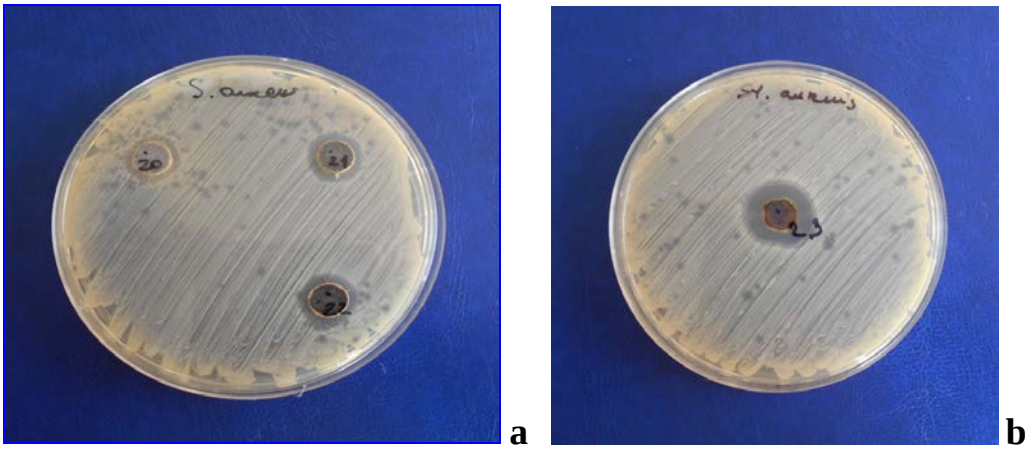
The substrates used for the growth of microorganisms were nutrient agar for bacteria and Sabouraud maltose agar (Torlak, Belgrade) for fungi. Substrates were sterilized for 15 minutes by autoclaving at 121°C under a pressure of 110 kPa. A 0.1cm3 of the proper inoculum culture was added to 10cm^3 of substrate and poured into Petri dishes. The samples were placed on the inoculated surface of the substrate. The incubation was carried out for 24 hours at 37°C for the bacteria and for 48 hours at 25°C for the fungi. After incubation, the growth inhibition zones were measured and values were expressed in mm. The presence of inhibition zone indicates the antimicrobial activity of the samples.

Results

The results have shown that the control sample (*Sample* 23) as well as the samples of silver impregnated acrylics resins (*Samples* 20-22) exhibit antibacterial activity. The growth zone inhibition of Staphylococcus aureus (*Figure* 1) in the culture medium is proportional to the concentration of silver nanoparticles in the cold curing acrylic resin (*Table* 1). Test samples did not inhibit the growth of Candida albicans in the medium (*Figure 2*).

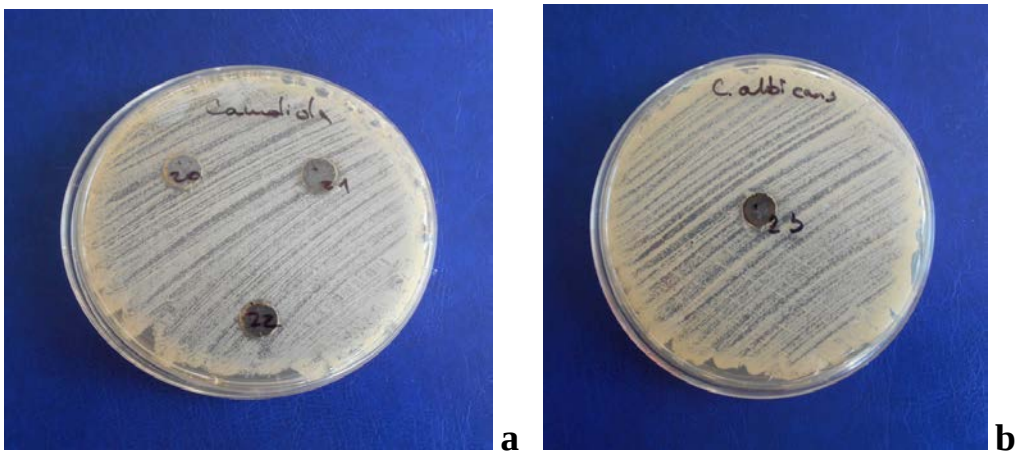
Tabela 1. Antimikrobna analiza akrilnih uzoraka smole natopljene AgNPs
Table 1. Antimicrobial analysis of acrylic resin samples impregnated with AgNPs

Ime uzorka **Sample name**	**% Ag u praškastoj supstanci** **Ag % in powder sample**	**Težina (m)** **Mass (m)**	**Staphylococcus aureus Inhibiciona zona Staphylococcus aureus inhibition zone**	**Candida albicans Inhibiciona zona Candida albicans inhibition zone**
Sample 20 Uzorak 20	2 %	0.02804g	12.62 mm	/
Sample 21 Uzorak21	5%	0.02828g	13.01 mm	/
Sample 22 Uzorak 22	10%	0.02833 g	13.28 mm	/
Sample 23 Uzorak 23	100%	0.01400 g	17.30 mm	/



a **b**

Slika 1. Zona inhibicije Staphylococcus aureus ATCC 25923 za uzorke 20-22 (a) i za kontrolni uzorak 23 (b).
Figure 1. Staphylococcus aureus ATCC 25923 growth inhibition zone for samples 20-22 (a) and for the control sample 23 (b).



a **b**

Slika 2. Zona inhibicije Candide albicans ATCC 2091 za uzorke 20-22 (a) i za kontrolni uzorak 23 (b).
Figure 2. Candida albicans ATCC 2091 growth inhibition zone for samples 20-22 (a) and for the control sample 23 (b).

Diskusija

U poređenju sa drugim metalima koji su u sastavu nanočestica, srebro u niskim koncentracijama pokazuje slab toksični efekat na ćelije čoveka [12-15]. Istraživanja su pokazala da je za antimikrobnu aktivnost zaslužan pozitivno naelektrisani Ag+ jon, koji omogućava elektrostatsko vezivanje negativno naelektrisane bakterijske ćelijske membrane i pozitivno naelektrisanih nanočestica [16,17]. Kwakye-Awauach i sar. su zaključili da se antimikrobni mehanizam bazira na interakciji Ag i tiol enzimskih grupa u metabolizmu bakterijske ćelijske membrane, što uzrokuje ćelijsku smrt [18].

Usna duplja je naseljena velikim brojem mikroorganizama koji u određenim uslovima mogu dovesti do različitih oralnih infekcija. Nošenje zubnih nadoknada, a samim tim i mobilnih proteza, menja uslove usne duplje, te nedvosmisleno utiče i na oralnu floru. Akrilatni materijali, a posebno hladno polimerizovani akrilati, pogodan su materijal za naseljavanje bakterija i gljivica, pa je njihova dobra higijena imperativ. Imajući u vidu starosnu i socijalnu strukturu nosioca mobilnih zubnih proteza, poboljšanje bioloških svojstava akrilata značajno bi smanjilo moguće komplikacije u vidu infekcija i zapaljenskih reakcija.

Cilj istraživanja bio je oplemenjivanje hladno polimerizovanog akrilatnog materijala AgNPs radi poboljšanja njegovog biološkog kvaliteta. Pošlo se od pretpostavke da akrilatni materijal impregniran srebrom u ustima deluje fungicidno i antimikrobno, te se od isključivo mehaničke nadoknade dobija profilaktičko i eventualno terapijsko sredstvo. Ispitivane su različite koncentracije AgNPs dodatog prahu hladno polimerizovanog akrilata u kontaku sa Staphylococcus aureusom i Candidom albicans. Rezultati su ukazali na pozitivni antimikrobni efekat impregniranog materijala na Staphylococcus aureus, jer je inhibitorni efekat bio veći sa porastom koncentracije AgNPs. Dobijeni rezultati su u saglasnosti sa nalazima Castro i sar. koji su pokazali da dodatak 5% i 10% β-AgVO3 značajno smanjuje metaboličku aktivnost Pseudomonasa aeruginosa i Staphylococcusa aureusa u oba tipa polimerizacije akrilata za bazu zubne proteze [13-19]. Slane i sar. su zaključili da AgNPs dodate koštanom PMMA cementu imaju snažnu antimikrobnu aktivnost u odnosu na Staphylococcus aureus [20].

Discussion

Compared with other metals that are a part of nanoparticles, silver in low concentrations shows weak toxic effects on human cells [12-15]. Studies have shown that the positively charged Ag+ ion is responsible for its antimicrobial activity, which allows the binding of the negatively charged electrostatic bacterial cell membrane and the positively charged nanoparticles [16,17]. Kwakye-Awauach et al. concluded that the mechanism of antimicrobial activity is based on the interaction of Ag and the thiol enzyme group in the metabolism of the bacterial cell membrane, thus causing cell death [18].

The oral cavity is populated by a large number of microorganisms which under certain conditions can lead to a wide variety of oral infections. Wearing dentures, and therefore removable dentures, changes the conditions of the oral cavity, and undoubtedly affects the oral flora. The acrylic materials, such as cold polymerized acrylates, are suitable materials for colonization of bacteria and fungi and as such their good hygiene is an imperative. Bearing in mind the age and social structure of denture holders, the improvement of the biological properties of acrylates would significantly reduce the possible complications in the form of infection and inflammatory reactions.

The research objective was refining cold polymerized acrylic material AgNPs to improve its biological quality. It was presumed that the resin material impregnated with silver acts in the mouth as fungicidal and antimicrobial, and that the purely mechanical compensation provides prophylactic and possibly therapeutic agent. Different concentrations of the tested AgNPs were added to the powder component of the cold polymerized acrylates, and such acrylates were in contact with Staphylococcus aureus and Candida albicans. The results showed a positive effect of antimicrobial activity of the impregnated material on Staphylococcus aureus because the inhibitory effect was greater with the increasing concentration AgNPs. The results are consistent with the findings of Castro et al. who have shown that the addition of 5% and 10% β-AgVO3 significantly reduces the metabolic activity of the Pseudomonas aeruginosa and Staphylococcus aureus in both types of polymerization of the acrylate denture base [13-19].

Khurana i sar. ukazuju na značaj smanjenja veličine partikle AgNPs u inhibiciji rasta Staphylococcus aureusa [21]. Sa druge strane, istraživanja Morrison i sar. nisu pokazala uticaj kombinacije AgNPs i praha akrilata na formiranje biofilma Staphilococcus pseudointermedius [22].

Dobijeni rezultati nisu dokazali fungicidni efekat AgNPs. Literaturno dostupni podaci jasno opisuju dejstvo AgNPs na kolonije kandide u ustima pacijenta, što uslovljava ponavljanje istraživanja istim metodološkim postupkom, kao i uvođenjem novih metoda [23,24].

S obzirom na ograničenost ovog istraživanja ne možemo doneti jasne sudove o antimikrobnim svojstvima impregniranih akrilatnih materijala. Njihov dokazani inhibitorni efekat na rast *Staphilococcus aureusa* podsticaj je za buduća istraživanja uticaja impregnacije AgNPs toplo i hladno polimerizovanih akrilata na različite vrste mikroorganizama koji naseljavaju usnu duplju.

Zaključak

Srebrne nanočestice dodate hladno-polimerizujućem akrilatu pokazale su antibakterijsku aktivnost. Širenje njihovog antimikrobnog spektra delovanja, kao i njihova potencijalna klinička primena , biće predmet budućih istraživanja.

Slane et al. concluded that the AgNPs added to the PMMA bone cement have a strong antimicrobial activity with respect to Staphylococcus aureus [20]. Khurana et al. point to the importance of reducing the size of AgNPs particles to inhibit the growth of Staphylococcus aureus [21]. On the other hand, the research of Morrison et al. did not confirm the effect of the combination of AgNPs and powder acrylate component on the biofilm formation by Staphylococcus pseudo-intermedius [22].

The results did not prove the fungicidal effect of AgNPs. Literary available data clearly describe the effect AgNPs on the colonization of Candida in the mouth, dictating the necessity for the repetition of the same methodological research process, as well as the introduction of new research methods [23,24].

Given the limitations of this study, we cannot make a clear judgment on the antimicrobial properties of resin impregnated materials. Their proven inhibitory effect on the growth of *Staphylococcus aureus* is incentive for future research on the impact of impregnation of hot and cold polymerized acrylates with AgNPs on different types of microorganisms that inhabit the oral cavity.

Conclusion

Silver nanoparticles in cold curing acrylic resin demonstrated antibacterial activity. Expanding their antimicrobial spectrum of activity and their potential clinical application will be the subject of the future research.

LITERATURA / REFERENCES

1. Marsh PD, Martin MV. Oral microbiology. 5th ed. London, UK: Butterworth-Heinemann; 2010.
2. Marsh PD, Bradshaw DJ. Dental plaque as a biofilm. J Ind Microbiol1995;15:169–75
3. Rodriguez LS, Paleari AG, Giro G, Oliveira Junior NM, Pero AC, Compagnoni MA. Chemical characterisation and flexural strength of a denture base acrylic resin with monomer 2-tert-butylaminoethyl methacrylate. J Prosthodont. 2013;22:292-7.
4. Diaz-Arnold AM, Vargas MA, Shaull KL. Flexural and fatigue strengths of denture base resin. J Prosthet Dent. 2008;100:47–51.
5. Valentiti F, Luz MS, Boscato N, Pereira-Cenci T. Biofilm formation on denture liners in a randomised controlled in situ trial. J Dent. 2013;41:420-7.
6. Wady AF, Machado AL, Zucolotto V, Zamperini CA, Berni E, Vergani CE. Evaluation of Candida albicans adhesion and biofilm formation on a denture base acrylic resin containing silver nanoparticles. J Appl Microbiol. 2012;112:1163-72.
7. B Kwakye-Awuah, C Williams, MA Kenward, I Radecka. Antimicrobial action and efficiency of silver-loaded zeolite X. . J Appl Microbiol. 2008; 104:5, 1516-1524.
8. Jeong SH, Yeo SY, Yi CS. The effect of filler particle size on the antibacterial properties of compounded polymer/silver fibers. J Mater Sci. 2005;40:5407–5411.
9. Bürgers, R., Eidt, A., Frankenberger, R., Rosentritt, M., Schweikl, H., Handel, G., et al. (2009). The anti-adherence activity and bactericidal effect of microparticulate silver additives in composite resin materials. Archives of Oral Biology, 54, 595– 601. Bershtein, V.
10. Yang, S. X., Zhang, Y. H., Yu, J. M., Zhen, Z. C., & Huang, T. Z. (2014). Antibacterial and mechanical properties of honeycomb ceramic materials incorporated with silver and zinc. Materials &. Design, 59, 461–465.
11. J. A. Kiehlbauch, G. E. Hannett, M. Salfinger, W. Archinal,C. Monserrat, C. Carlin, Use of the National Committee forClinical Laboratory Standards Guidelines for Disk diffusionsusceptibility testing in New York State Laboratories,Journal of Clinical Microbiology, 38(9) (2000) 3341 - 3348.
12. Allaker RP, Memarzadeh K. Nanoparticles and the control of oral infections. Int J Antimicrob Agents. 2014; 43:95– 104.
13. de Castro DT, Holtz RD, Alves OL, Watanabe E, Valente ML, da Silva CHL, dos Reis AC. Development of a novel resin with antimicrobial properties for dental application.J Appl Oral Sci. 2014;22(5):442-9
14. Bürgers R, Eidt A, Frankenberger R, Rosentritt M, Schweikl H, Handel G, et al. The anti-adherence activity and bactericidal effect of microparticulate silver additives in composite resin materials. Arch Oral Biol.2009; 54,:595– 601.
15. Yang SX, Zhang YH, Yu JM, Zhen ZC, Huang TZ. Antibacterial and mechanical properties of honeycomb ceramic materials incorporated with silver and zinc. Materials &. Design 2014; 59: 461–465.
16. Kiehlbauch JA, Hannett GE, Salfinger M, Archinal W, Monserrat C, Carlin C. Use of the National Committee forClinical Laboratory Standards Guidelines for Disk diffusionsusceptibility testing in New York State Laboratories. Journal of Clinical Microbiology 2000; 38(9): 3341 - 3348.
17. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, et al. Antimicrobial effects of silvernanoparticles. Nanomedicine 2007;3:95–101.
18. Kwakye-Awuah B, Williams C, Kenward MA, Radecka I. Antimicrobial action and efficiency of silver-loaded zeolite. J Appl Microbiol. 2008; 104(5): 1516-1524.
19. de Castro DT, Valente ML1 da Silva CH, Watanabe E, Siqueira RL, Schiavon MA, Alves OL, dos Reis AC. Evaluation of antibiofilm and mechanical properties of new nanocomposites based on acrylic resins and silvervanadate nanoparticles.Arch Oral Biol. 2016; 67:46-53.
20. Slane J, Vivanco J, Rose W, Ploeg HL, Squire M. Mechanical, material, and antimicrobial properties of acrylic bone cement impregnated with silver nanoparticles.Mater Sci Eng C Mater Biol Appl. 2015; 48:188-196.
21. Khurana C, Vala AK, Andhariya N, Pandey OP, Chudasama B. Antibacterial activity of silver: the role of hydrodynamic particle size at nanoscale.J Biomed Mater Res A. 2014; 102(10):3361-3368.
22. Morrison S, Singh A, Rousseau J, Walker M, Nazarali A, Crawford E, Brisson B, Sears WC, WeeseJS. Impact of polymethylmethacrylate additives on methicillin-resistant Staphylococcus pseudintermedius biofilm formation in vitro.Am J Vet Res. 2015; 76(5):395-401.
23. Lara HH, Romero-Urbina DG, Pierce C, Lopez-Ribot JL, Arellano-Jimenez MJ, Jose-Yacaman M. Effect of silver nanoparticles on Candidaalbicans biofilms: an ultrastructural study. J Nanobiotechnol 2015; 13:91-103.
24. Acosta-Torres LS, Mendieta J, Nunez-Anita RE, Cajero-Juanez MC, Castano VM. Int J Nanomedicine. 2012; 7: 4777–4786.

Primljen/ Recived on : 20.02.2017.
Revidiran/ Revised on : 13.03.2017.
Prihvaćen/ Accepted on : 27.03.2017.

ORGINALNI RAD
KLINIČKA STUDIJA
ORIGINAL ARTICLE
CLINICAL STUDY
doi:10.5937/asn1775703P

ŠIRINA PRIPOJNE GINGIVE I NJENA VARIJABILNOST KOD OSOBA SA ZDRAVIM PARODONTALNIM STATUSOM

THE WIDTH OF THE ATTACHED GINGIVA AND ITS VARIABILTY IN PEOPLE WITH HEALTHY PERIODONTAL STATUS

Ana S. Pejčić [1], Radmila R. Obradović [1], Dimitrije S. Mirkovic[2]

[1]UNIVERZITET U NIŠU, MEDICINSKI FAKULTET, KLINIKA ZA STOMATOLOGIJU, ODELJENJE ZA PARODONTOLOGIJU I ORALNU MEDICINU, NIŠ, SRBIJA
[2] PRIVATNA STOMATOLOŠKA ORDINACIJA "MIRKOVIC", NIŠ, SRBIJA

[1]UNIVERSITY OF NIŠ, FACULTY OF MEDICINE, CLINIC OF DENTISTRY, DEPARTMENT OF PERIODONTOLOGY AND ORAL MEDICINE, NIŠ, SERBIA
[2] PRIVATE DENTAL PRACTICES "MIRKOVIC", NIŠ, SERBIA

Sažetak

Uvod: *Gingiva je deo sluzokože koja pokriva avleolarne grebene vilica i okružuje vratove zuba. Anatomske karakteristike gingive su veoma važne u planiranju lečenja oboljenja parodonta. Pripojna gingiva je važna za održavanje parodonta u zdravom stanju. Ovom studijom merene su normalne vrednosti širine pripojne gingive kod parodontalno zdravih osoba.*
Cilj *rada bio je procena širinepripojne gingive različitim metodama.*
Materijal i metode: *Širina pripojne gingive merena je korišćenjem parodontalne sonde kod parodontalno zdravih osoba. U zavisnosti od starosti pacijenata, formirane su četiri grupe (I - ≤ 14 god; II - 15-30 god; III - 31-45 god i IV - 46-60 god.). Deskriptivna statistička analiza urađena je za određivanje srednjih vrednosti pripojne gingive , koje će predstavljati normalne vrednosti širine pripojne gingive za osobe sa zdravim parodontom.*
Rezultati*: Kod ispitanika starosti od 15-30 godina, nađena je najveća širina pripojne gingive, kao i kod osoba ženskog pola u odnosu na mušku grupu ispitanika. Srednje vrednosti širine pripojne gingive varirale su u zavisnosti od područja usne duplje: najveća širina pripojne gingive zabeležena je u predelu gornjih centralnih sekutića, dok je najmanja širina zabeležena u predelu prvih molara gornje i donje vilice.*
Zaključak: *Širina pripojne gingive varira u odnosu na starost i pol osobe, kao i od mesta u usnoj duplji.*

Ključne riječi: parodont, pripojna gingiva, zdravlje

Address for correspondence:
Ana Pejčić DDS, MSc, PhD
Nade Tomić 3/5
18000 Niš, Serbia
Phone:381/642572178
E-mail:dranapejcic@hotmail.com

Abstract

Background*: Gingiva is part of the mucous membrane that covers the alveolar ridges of the jaw and surrounds the necks of the teeth. Anatomical features of the gingiva are very important in planning the treatment of periodontal disease. The attached gingiva is important for maintaining a healthy periodontal condition. This study measured the normal value width of the attached gingiva in periodontal healthy subjects.*
The aim *was estimating the width of the attached gingiva by warious methods.*
Material and Methods*: The width of the attached gingiva was measured using a periodontal probe in periodontally healthy subjects. The measurement was performed in the Department of Periodontology and Oral Medicine Clinic of Dentistry, Faculty of Medicine University of Nis. Descriptive statistical analysis was performed to determine the mean values of the attached gingiva, which will represent the normal value width of the attached gingiva for people with healthy periodontium.*
Results: *The greatest width of the attached gingiva was found in subjects aged 15-30 years, primarily in the female population. The mean value of the width of the attached gingiva varied depending on the area in the mouth: the maximum width of the attached gingiva was noted in the area of the upper central incisors, while the lowest recorded value was in the area of the first molars, both in the upper and lower jaw.*
Conclusion: *The width of the attached gingiva varies according to the age and sex of the person, and according to the site in the oral cavity.*

Key words: periodontium, attached gingiva, health

Uvod

Pripojna gingiva je jedan od najvažnijih anatomskih i funkcionalnih delova potpornog aparata zuba. Iako u literaturi nema dovoljno podataka o ulozi pripojne gingive u održavanju parodontalnog zdravlja, odsustvo ili mala širina pripojne gingive može dovesti do bržeg širenja zapaljenja kod osoba koje ne održavaju dobru oralnu higijenu [1].

Zdrava pripojna gingiva je svetloružičaste boje sa površinom koja je tačkasta. Pripojna gingiva, zajedno sa palatinalnom sluzokožom, spada u mastikatorni tip sluzokože. Iz tih razloga, prekrivena je epitelom koji keratinizira. Deo pripojne gingive pripojen je za cement zuba, dok je veći deo pripojen za alveolarnu kost.

Širina pripojne gingive predstavlja rastojanje od donje ivice slobodne gingive u nivou dna gingivalnog sulkusa do mukogingivalne linije u vestibulumu [1]. Uprkos raznim mišljenjima u vezi sa adekvatnom količinom keratinizovanog tkiva za održavanje zdravlja parodonta, mukogingivalna linija služi kao važno kliničko obeležje u proceni parodonta [2]. Mukogingivalna linija je diskretna linija koja predstavlja granicu između pokretne i nepokretne sluzokože prilikom pasivnog kretanja usana i obraza [3]. Metode za lociranje mukogingivalne linije su vizuelna metoda (VM), funkcionalna metoda (FM) i Šilerova metoda (ŠM) [4].

Vizuelna metoda se zasniva na razlici u boji između gingive i alveolarne sluzokože [5]. U funkcionalnoj metodi se mukogingivalna linija ocenjuje kao granica između pokretnog i nepokretnog tkiva gde se mobilnost tkiva, utvrđuje korišćenjem parodontalne sonde, koja je pozicionirana laganim pritiskom horizontalno u predvorju usana prema ivici gingive [3]. Mukogingivalna linija može se proceniti i vizuelno posle bojenja mukogingivalnog kompleksa Lugolovim rastvorom (jodna proba). Alveolarna sluzokoža razlikuje se od keratinizovane gingive u sadržaju glikogena, kisele fosfataze i nespecifične esteraze i povećanoj količini elastičnih vlakana, što rezultira u pozitivnoj jodnoj reakciji [6-9].

Pripojna gingiva koja je keratinizovana ne sadrži glikogen, tako da površinski sloj daje negativnu jodnu reakciju. Tako, Lugolov rastvor prebojava samo alveolarnu sluzokožu i jasno razgraničava mukogingivalnu liniju.

Introduction

The attached gingiva is one of the most important anatomical and functional parts of the tooth supporting apparatus. Although there is insufficient literary data on the role of the attached gingiva in maintaining periodontal health, the absence or small width of the attached gingiva may lead to faster spread of inflammation in people who do not maintain good oral hygiene [1].

Healthy attached gingiva is of light pink color with an area that is dotted. Attached gingiva, together with the palatal mucosa is one of the masticatory tips of mucous membranes. For these reasons, it is covered by keratinized epithelium. Part of the attached gingiva was annexed to the cement teeth, while the larger part was annexed to the alveolar bone.

The width of the attached gingiva is the distance from the bottom edge of the free gingiva in the bottom of the gingival sulcus to the gingival line in the vestibulum [1]. Despite various opinions regarding the appropriate amount of keratinized tissue to maintain periodontal health, mucogingival line serves as an important landmark in the clinical assessment of periodontium [2]. Mucogingival line is a discrete line representing the boundary between mobile and immobile mucous membranes in the passive movement of the lips and cheeks [3]. Methods for locating gingival lines are visual method (VM), functional (FM) and Shiler'method (ŠM) [4].

Visual method is based on the difference in color between the gingival and alveolarmucous membrane [5]. In the functional method, the mucogingival line is estimated as a border between the movable and immovable tissue where the mobility of tissue is determined using a periodontal probe that is positioned horizontally by pressing gently in the lobby of the lips to the edge of the gingiva [3]. Mucogingival line can be assessed visually after staining the mucogingival complex with Lugol solution (iodine test). Alveolar mucosa differs from the gingival curettage in glycogen, acid phosphatase, and the non-specific esterase, and an increased amount of elastic fibers, resulting in a positive reaction on iodine[6-9]. The attached gingiva, which is keratinized, does not contain glycogen so that the surface layer provides a negative iodine reaction. Thus, the Lugol's solution stains only the alveolar mucosa and clearly demarcates the gingival line.

Širina pripojne gingive se znatno razlikuje i kreće se od jedan do devet milimetara. Širina se razlikuje u predelu pojedinih zuba, šira je u gornjoj vilici nego u donjoj i uža je kod mlečnih zuba [10].

Povećanje širine pripojne gingive ima veliku ulogu u parodontalnoj plastičnoj hirurgiji. Malo je studija koje su ispitivale širinu pripojne gingive kod parodontalno zdravog stanovništva. Merenje širine pripojne gingive će pomoći u proceni rizika za pojavu i pogoršanje parodontalnih oboljenja kod parodontalno zdrave populacije.

Ciljevi ove studije bili su da se proceni širina pripojne gingive u punom zubnom nizu u ustima i razlike između vizuelne metode, određivane uz pomoć parodontalne sonde i vizuelne metode, određivane nakon Šilerove probe.

Materijal i metode

U ovom istraživanju učestvovalo je ukupno 120 osoba odabranih iz Službe za parodontologiju i oralnu medicinu Klinike za stomatologiju Medicinskog fakulteta u Nišu. Za odabir učesnika korišćena je neproporcionalna slojevita nasumična metoda. Uz ovu metodu, primenjivali su se i kriterijumi uključivanja i isključivanja u istraživanje. Pacijenti starosti do 60 godina, sa dobrim opštim zdravljem, zdravim gingivalnim tkivom (nema gubitka pripoja) i oni koji nisu imali neku vrstu parodontalnog tretmana u poslednjih 6 meseci, bili su uključeni u studiju. Iz studije su isključene trudnice i dojilje, zatim pacijenti sa sistemskim bolestima i oni koji su uzimali lekove koji mogu imati uticaj na stanje gingive.

Pacijenti su obavešteni o protokolu istraživanja i oni koji su pristali na ispitivanje, potpisali su pristanak. U zavisnosti od starosti pacijenata, formirane su sledeće grupe

I	≤ 14 godina	III	31-45 godina
II	15-30 godina	IV	46-60 godina.

Širina pripojne gingive vizuelnom metodom merena je uz primenu parodontalne sonde Michigen 0 i stomatološkog ogledalceta pri veštačkoj svetlosti stomatološke stolice.

Drugi način izvođenja vizuelne metode merenja širine pripojne gingive bila je primena rastvora joda (Lugol rastvor) koji je pripremljen razblaživanjem 2 g kalijum jodida i 1 g kristala joda u 60 ml destilovane vode [11].

The width of the attached gingiva varies considerably, ranging from one to nine millimeters. The width varies in the area of individual tooth; it is wider in the upper jaw than in the lower one and is narrower near the milk teeth [10].

Increasing the width of the attached gingiva has a great role in periodontal plastic surgery. There are very few studies that have examined the width of the attached gingiva in periodontal healthy population. Measuring the width of the attached gingiva will assist in assessing the risk of the emergence and worsening of periodontal disease in a periodontally healthy population.

The objectives of this study were to estimate the width of the attached gingiva in full dental arch in the mouth and to assess the differences between visual methods determined with the help of periodontal probes and visual methods determined after Schiller's probe.

Material and Methods

This study involved a total of 120 people selected from the Department of Periodontology and Oral Medicine, Dental Clinic School of Medicine in Nis. For the selection of participants, a disproportionate stratified random method was used. Along with this method, the criteria for inclusion and exclusion were also applied. Patients up to the age of 60 years with good general health, healthy gingival tissue (no loss of attachment) and those who did not have some kind of periodontal treatment in the last six months were included in the study. Pregnant and lactating women, patients with systemic diseases, and those taking drugs that may affect the condition of the gingiva were excluded from the study.

Patients were informed about the study protocol, and those who agreed with the examination terms submitted the written informed consent. Depending on the age of the patient, the following groups were formed:

I	≤ 14 years	III	31-45 years
II	15-30 years	IV	46-60 years.

The width of the attached gingiva was measured with the use of periodontal probes Michigan 0 and dental mirror artificial light in the dental chair.

Another way of measuring the width of the attached gingiva was the application of iodine solution (Lugol's Solution), which was prepared by diluting 2 g of potassium iodide

Merenje širine pripojne gingive vršilo se na centralnoj poziciji bukalne površine svih centralnih sekutića, očnjaka, prvih premolara i prvih molara, na ukupno 16 zuba. Ceo postupak je sproveden od strane jednog ispitivača.

Da bi se procenila širina pripojne gingive, mukogingivalna linija je označena na sledeći način:

• vizuelnom metodom – 1. način (Slika 1)

• vizuelnom metodom nakon Šilerove probe – vrši se premazivanje pamučnom paletom blagim pritiskom tkiva gingive i alveolarne sluzokože do naglog razgraničenja između keratinizovane gingive i alveolarne mukoze (Slika 2).

Širina keratinizovane gingive je merena kao udaljenost od gingivalne ivice do mukogingivalne linije. Dubina sulkusa je merena kao udaljenost od gingivalne ivice do donje ivice slobodne gingive (dna gingivalnog sulkusa). Sa ovim vrednostima, širina pripojne gingive bila je izračunavana kao razlika dubine sulkusa i širine keratinizovanog tkiva.

and 1 g of a crystal of iodine in 60 ml of distilled water [11].

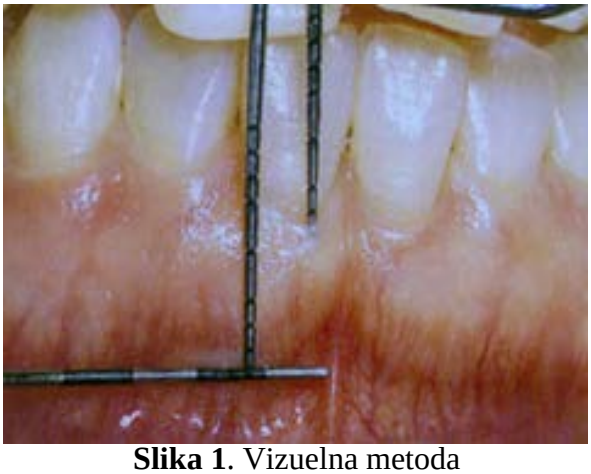
Measuring the width of the attached gingiva was performed in the central position of the buccal surface of the central incisors, canines, first premolars and first molars, i.e. a total of 16 teeth. The entire procedure was carried out by one examiner.

To assess the width of the attached gingiva, mucogingival line is indicated as follows:

• visual method - Method 1 (Figure 1)

• visual method after a Schiller's probe – staining is performed using a coating cotton palette, applying a slight pressure over the gingival and alveolar mucous membrane to a sharp demarcation between the subgingival curettage and alveolar mucosa (Figure 2).

The width of subgingival curettage is measured as the distance from the gingival edge to the mucogingival line. Sulcus depth was measured as the distance from the gingival edge to the bottom edge of the free gingiva (bottom of the gingival sulcus). With these values, the width of attached gingiva was calculated as the difference sulcus depth and width keratinized tissue.



Slika 1. Vizuelna metoda
Figure 1. Visual method

Slika 2 Vizuelna metoda nakon Šilerove probe
Figure 2. Visual method after a Shiler's method

Statistička obrada

Svi prikupljeni podaci su obrađeni u programskom paketu SPSS 15.0. Rađena je srednja vrednost i standardna devijacija dobijenih rezultata. Podaci pokazuju homogenost i razlike između analiziranih grupa korišćenjem ANOVA testa . Razlika između grupa je analizirana korišćenjem post-hoc testa. Kriterijum za statističku značajnost je prihvaćen na nivou verovatnoće $p < 0,05$.

Rezultati

Ukupno 120 ispitanika je učestvovalo u ovom istraživanju, od kojih je 74 (61,67 %) bilo ženskog pola.

Ženske ispitanice su imale veću prosečnu širinu pripojne gingive (3,65 mm) od muških, koji su imali prosek od 2,42 mm (Tabela 1).

Maksilarni centralni sekutići imali su najveću širinu pripojne gingive sa prosekom od 3,57 mm, dok su mandibularni prvi molari imali najmanju širinu pripojne gingive (2,35 mm). Centralni sekutići u mandibuli imali su širinu 2,48 mm, maksilarni premolari 2,99 mm, mandibularni premolari 2,67 mm, dok su maksilarni prvi molari bili sa širinom od 2,58 mm (Tabela 2).

Procena širina pripojne gingive u različitim starosnim grupama vizuelnom metodom na 1. način otkrila je da se širina gingive povećava sa starenjem, gde je prosečna širina na maksilarnim zubima u prvoj starosnoj grupi (≤14 godina) bila 1,73 mm, sa povećanjem od 2,25 do 2,89 u drugoj grupi (15-30 godina) i trećoj starosnoj grupi (31-45 godina), dok je u starosnoj grupi od 46-60 godina bila 3,25 mm.

Slično tome, kod donjih zuba u prvoj starosnoj grupi (≤14 godina), prosečna širina bila je 1,35 mm, sa povećanjem na 2,13 mm u drugoj i 2,10 mm u trećoj starosnoj grupi (15-45 godina) i 2,45 mm u starosnoj grupi od 45-60 godina (Tabela 3). Procena širine pripojne gingive u različitim starosnim grupama vizuelnom metodom nakon Šilerove probe pokazala je u proseku širinu pripojne gingive od 2,11 mm u prvoj starosnoj grupi (≤14 godina) do 3,25 mm u starosnoj grupi od 45-60 godina starosti za gornju vilicu. Kod donjih zuba, srednja vrednost širine pripojne gingive kod prve starosne grupe (≤14 godina) bila je 1,64 mm, dok je u najstarijoj starosnoj grupi od 45-60 godina bila 2,48 mm (Tabela 2).

Statistical analysis

All collected data was analyzed by SPSS 15.0. The mean value and standard deviation of the results were determined. The data show homogeneity and differences between the groups analyzed using ANOVA test. The difference between groups was analyzed using post-hoc test. The criteria for statistical significance was accepted at the probability level of $p < 0.05$.

Results

A total of 120 respondents participated in the survey, of which 74 (61.67%) were female.

Female respondents had a greater average width of the attached gingiva (3.65 mm) than men in whom the average width of width of the attached gingiva was 2.42 mm (Table 1).

The maxillary central incisors had a maximum width of the attached gingiva with an average of 3.57 mm, while the mandibular first molars had a minimal width of the attached gingiva (2.35 mm). The width of the central incisors in the mandible was 2.48 mm, 2.99 mm of the maxillary premolars, 2.67 mm of the mandibular premolars, while the width of the maxillary first molars was 2.58 mm (Table 2).

Visual assessment of the attached gingiva width in different age groups using the method 1 revealed that the width of the gingiva increases with age, where the average width of the maxillary teeth in the first age group (≤14 years of age) was 1.73 mm, with an increase of 2.25 to 2.89 in the second group (15-30 years) and the third age group (31-45 years), while in the age group of 46-60 years it was 3.25 mm.

Similarly, in the lower teeth in the first age group (≤14 years) the average width was 1.35 mm, increasing to 2.13 mm in the second and 10.2 mm in the third age group (15-45 years) and 2.45 mm in the age group of 45-60 years (Table 3).

Assessment of the width of the attached gingiva in different age groups by visual method after Schiller's probe showed that the average width of the attached gingiva was 2.11 mm in the first age group (≤14 years) i.e. 3.25 mm in the age group of 45-60 years of age for the upper jaw. In the lower teeth, the mean width of the attached gingiva in the first age group (≤14 years of age) was 1.64 mm, while in the oldest age group of 45-60 years it was 2.48 mm (Table 2).

Ne postoji statistički značajna razlika u širini pripojne gingive obe vilice u starosnoj grupi (≤14 godina) bez obzira na metod koji se koristi za procenu (Tabela 5).

Poređenje dva različita metoda različitih zuba u starosnoj grupi od 15-30 godina nije otkrilo nikakve značajne razlike, iako je najširа zona pripojne gingive pronađena kod sekutića primenom obe metode (metoda 1 - 4,07, metoda 2 – 3,85) i najmanja zona širine u premolarnom regionu (metoda 1 – 1,92, metoda 2 – 2,07). Ovakve varijacije su slične i kod donjih zuba (Tabela 6).

Slične vrednosti maksimalne širine kod gornjih sekutića i kod donjih premolara uočeni su i u drugim starosnim grupama, odnosno u grupi od 31 ‑ 45 godina i grupi od 46 ‑ 60 godina, tako da nije uočena statistički značajna razlika između dve metode (Tabele 7 i 8).

There was no statistically significant difference in the width of the attached gingiva in both jaws in the age group (≤14 years), regardless of the method used for the assessment (Table 5).

Comparison of two different methods of different teeth in the age group of 15-30 years did not show any significant differences, though the widest zone of the attached gingiva was found for incisors by the application of both methods (method 1 - 4.07, method 2 - 3.85),whereas the minimum zone width was seen in the premolar region (method 1 - 1.92, method 2 - 2.07). Such variations are similar in the lower teeth as well (Table 6).

Similar values for the maximum width of the upper incisors and the lower premolars were observed in other age groups, i.e. in groups of 31 - 45 years and the group of 46-60 years, so there was not a significant difference between the two methods (Table 7 and 8).

Tabela 1. Širina pripojne gingive kod muškaraca i žena
Table 1. The width of the attached gingiva in men and women

pol sex	broj (procenat) number (percentage)	SV ± SD (mm)
ženski female	74 (61.67%)	3.654 ± 1.269
muški male	46 (38.33%)	2.426 ± 1.536

SV – srednja vrednost; SD – standardna devijacija
SV – mean; SD – standard deviation

Tabela 2. Širina pripojne gingive kod različitih zuba u vilicama
Table 2. The width of the attached gingiva in different teeth in the jaw

vrsta zuba type of teeth	SV ± SD (mm)
gornji centralni sekutići upper central incisors	3.57 ± 1.761
gornji očnjaci upper canines	3.28 ± 1.527
gornji prvi premolari upper first premolars	2.99 ± 1.929
gornji prvi molari upper first molars	2.58 ± 1.581
donji centralni sekutići lower central incisors	2.48 ± 1.370
donji očnjaci lower canines	2.18 ± 1.211
donji prvi premolari lower first premolars	2.67 ± 1.730
donji prvi molari lower first molars	2.35 ± 1.273

SV – srednja vrednost; SD – standardna devijacija
SV – mean; SD – standard deviation

Tabela 3. Procena širine pripojne gingive u različitim starosnim grupama vizuelnom metodom (način 1)
Table 3. Evaluation of the width of the attached gingiva in different age groups, using a visual method (method 1)

godine age	SV ± SD (mm)	F; p-vredn. F; p-value	Post-hoc test
gornji zubi upper teeth			
≤ 14	1.73 ± 0.77	F - 10.629 p < 0.001	II > I
15-30	2.25 ± 0.42		IV > I
31-45	2.89 ± 0.40		IV > II
46-60	3.25 ± 0.25		
donji zubi lower teeth			
≤ 14	1,35 ± 0.30	F - 6.398 p < 0.001	II > I
15-30	2.13 ± 0.42		III > I
31-45	2.10 ± 0.36		IV > I
46-60	2.45 ± 0.50		

SV– srednja vrednost; SD – standardna devijacija; F – vrednost signifakntnosti; p – statistički značajna razlika

SV– mean; SD – standard deviation; F – the value of significance; p – statistically significant difference

Tabela 4. Procena širine pripojne gingive u različitim starosnim grupama vizuelnom metodom nakon Šilerove probe (način 2)
Table 4. Visual assessment of the width of the attached gingiva in different age groups after a Schiller's probe (method 2)

godine age	SV ± SD (mm)	F; p-vredn. F; p-value	Post-hoc test
gornji zubi upper teeth			
≤ 14	2.11 ± 0.42	F - 8.558 p < 0.001	III > I
15-30	2.21 ± 0.52		IV > I
31-45	2.74 ± 0.49		IV > II
46-60	3.25 ± 0.63		
donji zubi lower teeth			
≤ 14	1.64 ± 0.38	F - 9.082 p < 0.001	II > I
15-30	2.08 ± 0.29		III > I
31-45	2.34 ± 0.38		IV > I
46-60	2.48 ± 0.41		

SV– srednja vrednost; SD – standardna devijacija; F – vrednost značajnosti; p – statistički značajna razlika

SV – mean; SD – standard deviation; F – the value of significance; p – statistically significant difference

Tabela 5. Poređenje dva različita metoda u starosnoj grupi ≤ 14 godina
Table 5. Comparison of two different methods in the age group ≤ 14 years

zubi teeth	vizuelna metoda visual method	SV ± SD (mm)	F; p-vredn. F; p-value
gornji upper	način 1 method 1	1.89 (0.42)	F – 0.78
	način 2 method 2	2.05 (0.43)	n.s.
donji lower	način 1 method 1	1.50 (0.43)	F – 0.84
	način 2 method 2	1.57 (0.35)	n.s.

SV– srednja vrednost; SD – standardna devijacija; F – vrednost značajnosti; p – statistički značajna razlika; n.s. – nije statistički značajna razlika

SV – mean; SD – standard deviation; F – the value of significance; p – statistically significant difference: n.s. – it is not statistically significant

Tabela 6. Poređenje dve različite metode u starosnoj grupi od 15 do 30 godina starosti
Table 6. Comparison of two different methods in the age group of 15-30 years

zubi teeth	vilica jaw	Vizuelna metoda Visual method	SV ± SD (mm)	F; p-vredn. F; p-value
sekutići incisors	gornja upper	način 1 method 1	4.07 ± 0,58	F – 0.82
		način 2 method 2	3.85 ± 0.84	n.s.
	donja lower	način 1 method 1	3.22 ± 0.73	F – 0.62
		način 2 method 2	3.12 ± 0.76	n.s.
očnjaci canines	gornja upper	način 1 method 1	3.80 ± 0,71	F – 0.72
		način 2 method 2	3.75 ± 0.98	n.s.
	donja lower	način 1 method 1	2.45 ± 0,48	F – 0.90
		način 2 method 2	2.65 ± 0.42	n.s.
premolari premolars	gornja upper	način 1 method 1	1.92 ± 0.65	F – 0.90
		način 2 method 2	2.07 ± 0.75	n.s.
	donja lower	način 1 method 1	1.50 ± 0.59	F – 0.84
		način 2 method 2	1.67 ± 0.38	n.s.
molari molars	gornja upper	način 1 method 1	2.67 ± 0.47	F - 0,97
		način 2 method 2	2.80 ± 0.57	n.s.
	donja lower	način 1 method 1	1.65 ± 0.65	F – 0.84
		način 2 method 2	1.92 ± 0.71	n.s.

SV– srednja vrednost; SD – standardna devijacija; F – vrednost značajnosti; p – statistički značajna razlika; n.s. – nije statistički značajna razlika

SV – mean; SD – standard deviation; F – the value of significance; p – statistically significant difference: n.s. – it is not statistically significant

Tabela 7. Poređenje dve različite metode u starosnoj grupi od 31 do 45 godina starosti
Table 7. Comparison of two different methods in the age group of 31-45 years

zubi teeth	vilica jaw	vizuelna metoda visual method	SV ± SD (mm)	F; p-vredn. F; p-value
sekutići incisors	gornja upper	način 1 method 1	3.30 ± 1.08	F – 0.23
		način 2 method 2	3.62 ± 0.71	n.s.
	donja lower	način 1 method 1	3.22 ± 0.98	F – 0.87
		način 2 method 2	3.05 ± 0.72	n.s.
očnjaci canines	gornja upper	način 1 method 1	2.80 ± 1.25	F – 0.15
		način 2 method 2	2.95 ± 0.95	n.s.
	donja lower	način 1 method 1	1.80 ± 1.06	F – 0.86
		način 2 method 2	1.70 ± 0.94	n.s.
premolari premolars	gornja upper	način 1 method 1	2.07 ± 0.65	F – 0.54
		način 2 method 2	2.32 ± 0.73	n.s.
	donja lower	način 1 method 1	1.52 ± 0.47	F – 0.24
		način 2 method 2	1.47 ± 0.31	n.s.
molari molars	gornja upper	način 1 method 1	2.22 ± 0.74	F – 0.75
		način 2 method 2	2.42 ± 0.88	n.s.
	donja lower	način 1 method 1	1.97 ± 0.31	F – 0.68
		način 2 method 2	2.10 ± 0.48	n.s.

SV– srednja vrednost; SD – standardna devijacija; F – vrednost značajnosti; p – statistički značajna razlika; n.s. – nije statistički značajna razlika

SV – mean; SD – standard deviation; F – the value of significance; p – statistically significant difference: n.s. – it is not statistically significant

Tabela 8. Poređenje dve različite metode u starosnoj grupi od 46 do 60 godina starosti
Table 8. Comparison of two different methods in the age group of 46-60 years

zubi teeth	vilica jaw	vizuelna metoda visual method	SV ± SD (mm)	F; p-vredn. F; p-vrednost
sekutići incisors	gornja upper	način 1 method 1	3.65 ± 1.08	F – 0.14
		način 2 method 2	3.30 ± 0.71	n.s.
	donja lower	način 1 method 1	3.05 ± 0.98	F – 0.23
		način 2 method 2	3.22 ± 0.72	n.s.
očnjaci canines	gornja upper	način 1 method 1	2.95 ± 1.25	F – 0.21
		način 2 method 2	2.80 ± 0.95	n.s.
	donja lower	način 1 method 1	1.70 ± 1.06	F – 0.84
		način 2 method 2	1.80 ± 0.94	n.s.
premolari premolars	gornja upper	način 1 method 1	2.32 ± 0.65	F – 0.52
		način 2 method 2	2.07 ± 0.73	n.s.
	donja lower	način 1 method 1	1.47 ± 0.47	F – 0.33
		način 2 method 2	1.52 ± 0.31	n.s.
molari molars	gornja upper	način 1 method 1	2.42 ± 0.74	F – 0.80
		način 2 method 2	2.22 ± 0.88	n.s.
	donja lower	način 1 method 1	2.10 ± 0.31	F – 0.06
		način 2 method 2	1.97 ± 0.48	n.s.

SV– srednja vrednost; SD – standardna devijacija; F – vrednost značajnosti; p – statistički značajna razlika; n.s. – nije statistički značajna razlika

SV – mean; SD – standard deviation; F – the value of significance; p – statistically significant difference: n.s. – it is not statistically significant

Diskusija

Procena širine pripojne gingive je od vitalnog značaja za procenu rizika parodoncijuma za pojavu oboljenja. U proceni širine pripojne gingive mukogingivalna linija služi kao važan anatomski orijentir, koja se može razgraničiti različitim metodama. Kako sugeriše Fasske i Morgenroth, tačna lokacija ove linije može se videti posle bojenja sluzokože različitim rastvorima (kao što je Lugolov jodni rastvor), koji omogućavaju određivanje tačne linije na kojoj se keratinizacija završava [12]. U cilju eliminacije razlika u sondiranju, sva merenja su vršena od strane jednog ispitivača.

Postoji veliki broj studija koje su ispitivale širinu pripojne gingive. Najčitanije studije koje su ispitivale širinu pripojne gingive su studije Boversa iz 1963.[13] i 1976. godine i Ainima iz 1976. godine [14].U ovom ispitivanju merenje pripojne gingive vršilo se pomoću periodontalne sonde slično kao Tenenbaum [15], kao i korišćenjem rastvora joda (Šilerova jodna proba) kao Talari [16], Ainamo [17] i Saario [18,19]. Bovers [13] je pronašao da širina pripojne gingive varira na različitim mestima usne duplje, kao što je nađeno i u ovom ispitivanju. Ainamo [14] je pronašao najveću širinu pripojne gingive u predelu maksilarnih inciziva slično rezultatima u našoj studiji. Najmanja širina u našoj studiji je bila u predelu prvog molara u mandibuli, dok je Ainamo [14] pronašao da je najmanja širina u predelu premolara u mandibuli. Ainamo [14] i Vincent [20] su utvrdili da se širina pripojne gingive povećava sa uzrastom , što je u saglasnosti sa rezultatima iz ovog istraživanja, koji pokazuju da je najveća širina pripojne gingive bila u drugoj starosnoj grupi (15-30 god.), dok je u srednjoj starosnoj grupi od 31-45 godina širina pripojne gingive bila najmanja (tabela 3).

Procena širine pripojne gingive u različitim starosnim grupama vizuelnom metodom otkrila je da se širina pripojne gingive povećava sa godinama, što je u saglasnosti sa autorima poput Ainama i Talarija [16] i Vinsent sa sar.[20]. Širina pripojne gingive varira u različitim oblastima u usnoj duplji i iznosi od 1-9 mm, [21] 1-4 mm, [22] 0-5 mm [23].

U ovoj studiji, opseg srednje širine pripojne gingive varira od 1 mm do 4 mm. Slične varijacije se vide u Boverovoj studiji, gde je najširа zona pripojne gingive pronađena

Discussion

The assessment of the width of the attached gingiva is vital to assess the risk for the occurrence of periodontal disease. For the assessment of the width of the attached gingiva, mucogingival line serves as an important anatomical landmark, which can be delineated by various methods. As suggested by Fasske and Morgenroth, the exact location of this line can be seen after staining the mucosa with different solutions (such as Lugol iodine solution) which allow accurate determination of the line on which the keratinization ends [12]. For the purpose of elimination of the difference in probing, all measurements were performed by a single examiner.

There is a large number of studies that have examined the width of the attached gingiva. The most read studies related to the examination of the width of the attached gingiva are those authored by Bowers in 1963 [13] and 1976 and Ainamo in 1976 [14]. In this study, the measurement of the attached gingiva was performed using a periodontal probe similar to Tenenbaum [15], and using a solution of iodine (Schiller's test) as Talari[16], Ainamo [17] and Saario [18,19] did. Bowers [13] found that the width of the attached gingiva varies in different areas of the oral cavity, as it was found in this study. Ainamo [14] found the maximum width of the attached gingiva in the area of the maxillary incisors. Similar results were found in this study. Minimum width in this study was in the part of the first molar in the mandible, while Ainamo [14] found that the minimum width was in the region of the premolars in the mandible. Ainamo [14] and Vincent [20] found that the width of the attached gingiva increases with age, which is consistent with the results of this research. These results show that the maximum width of the attached gingiva was in the second age group (15-30 yrs.), while in the middle age group of 31-45 years the width of the attached gingiva was the smallest (Table 3).

The assessment of the width of the attached gingiva in different age groups using a visual method revealed that the width of the attached gingiva increases with age which is in accordance with the results obtained by Ainamo and Talari[16] and Vincent et al. [20] The width of the attached gingiva varies in different areas of the oral cavity, ranging from 1-9 mm, [21] 1-4 mm, [22], 0-5 mm[23].

In this study, the extent of medium width of the attached gingiva can vary from 1 mm to 4 mm. Similar variations are seen in

u predelu sekutića , a najmanja u predelu premolara, bez obzira na metod koji se koristi u proceni [21].

Kategorizacija različitih tipova zuba urađena je samo u poslednje tri starosne grupe, dok učesnici prve starosne grupe ≤ 14 godina, imaju različitu konfiguraciju prisutnih zuba zbog prisustva mešovite denticije. U slučaju mešovite denticije ne može se komentarisati zbog različite prirode prisutnih zuba.

Rezultati ove studije su pokazali da nije bilo značajne razlike u širini pripojne gingive dobijene merenjem vizuelnom metodom ili korišćenjem vizuelne metode nakon izvedene Šilerove probe. Ovi rezultati su u skladu sa istraživanjem Guglielmoni et al. [24], dok Bernimoulin et al. [25] prijavljuje da izvođenje funkcionalne metode daje najveću širinu keratinizirane gingive. Osim toga, vizuelna metoda, nakon bojenja gingivalnog tkiva, dovodi do najmanjeg neslaganja vrednosti širine pripojne gingive u poređenju sa drugim metodama određivanja širine pripojne gingive.

Najširа je u predelu sekutića, zatim se sužava u predelu premolara, da bi se opet nešto širila u predelu molara. U pogledu širine pripojne gingive postoji simetrija sa leve i desne strane lica, dok razlika između polova ne postoji. Širina pripojne gingive je važna i za lekara i za pacijenta, jer utiče na prognozu oboljenja. Ako je pripojna gingiva uzana, prognoza oboljenja parodonta će biti nepovoljnija. Kod osoba koje imaju uzanu pripojnu gingivu, proces destrukcije koji nastaje u toku bolesti, brže dospeva do mukogingivalne linije. Tada dolazi do izražaja vuča preko pokretne sluzokože i daljeg napredovanja patološkog procesa. Vuča koja deluje odvaja ivicu gingive od zuba i omogućava lakše stvaranje i zadržavanje dentalnog plaka, sa svim neželjenim posledicama. Ovaj problem postaje izrazitiji ako se na to nadovežu i anomalije u mekom tkivu.

the Bover's study, where the widest zone of the attached gingiva was found in the area of incisors, and the lowest one in the region of the premolars, regardless of the method used in the assessment [21].

The categorization of different types of teeth was done only in the last three age groups, while the participants of the first age group ≤14 years had a different configuration of the present teeth due to the presence of mixed dentition. The width of the attached gingiva cannot be compared in cases of mixed dentition because of the different nature of the present teeth.

The results of this study showed that there was no significant difference in the width of the attached gingiva obtained by measuring using the visual method or using visual methods implemented after Schiller probe. These results are consistent with the research of Guglielmone et al., [24] while Bernimoulin et al. [25] reported that performing the functional method gives a maximum width of keratinized gingiva. In addition, the visual method after staining the gingival tissue leads to the smallest disagreements in the values of the attached gingiva width compared to other methods of determining the width of the attached gingiva.

It is the widest in the area of incisors, and then it narrows in the premolar area to expand again slightly in the area of the molars. In terms of the width of the attached gingiva, there is a symmetry with the left and right sides of the face, while the difference between the sexes does not exist. The width of the attached gingiva is important for physicians and patients because it affects the prognosis of the disease. If the attached gingiva is narrow, the periodontal disease prognosis will be worse. Considering people with narrow attached gingiva, the process of destruction that occurs in the course of the disease reaches faster a mucogingival line. Then, there is a marked traction over the movable mucous membrane and further progression of the pathological process. Traction separates the edge of the gingiva from the teeth and facilitates the creation and retention of dental plaque, with all the adverse consequences. This problem becomes more noticeable if this is combined with the anomalies in the soft tissue.

Zaključak

Procena širine pripojne gingive u punom zubnom nizu otkriva različite širine u različitim predelima usne duplje u predelu maksilarnih inciziva je zabeležena najveća širina, dok je najmanja širina u predelu donjih prvih molara. Starost pacijenata utiče na širinu pripojne gingive, dok je kod ženskih osoba zabeležena veća širina pripojne gingive.

Potrebna su dalja istraživanja kod parodontalno zdravih osoba sa različitih teritorija Srbije kako bi se utvrdilo da li i geografski položaj možda utiče na različite vrednosti širine pripojne gingive. Takođe je potrebno da se ova istraživanja izvrše na većem broju uzoraka, kako bi se dobio definitivan opseg koji bi se mogao definisati kao adekvatan ili ne, što olakšava lečenje oboljenja parodonta.

Conclusion

Assessment of the width of the attached gingiva in full dental arch reveals different widths in different areas of the oral cavity. The maximum width is in the area of the maxillary incisors, while the smallest width is in the area of the lower first molar. Patients' age affects the width of the attached gingiva, while the females had greater width of the attached gingiva.

Further research is needed regarding the periodontally healthy people from different parts of Serbia in order to determine whether the geographical position may affect the different values of width of the attached gingiva. It is also necessary to perform this research on a larger number of participants to obtain a definite scope that could be defined as adequate or not, making it easier to treat periodontal disease.

LITERATURA / REFERENCES

1. Newman MG, Takei HH, Klokkevold PR, Carranza FA Eds Carranza's Clinical Periodontology, 10th (eds.), 2006.
2. Wennström JL, Zucchelli G, Pini GP. Mucogingival therapy‐ periodontal plastic surgery. In: Lindhe J, Lang NP, Karring T, editors. Clinical Periodontology and Implant Dentistry. 5th ed. Oxford: Blackwell Munksgaard Publishers; 2008. p. 956‐ 7.
3. Hilming F, Jervoe P. Surgical extension of vestibular depth. On the results in various regions of the mouth in periodontal patients. Tandlaegebladet 1970; 74: 329‐ 343.
4. Guglielmoni P, Promsudthi A, Tatakis DN, Trombelli L. Intra‐ and inter‐ examiner reproducibility in keratinized tissue width assessment with 3 methods for mucogingival junction determination. J Periodontol 2001; 72: 134‐ 139.
5. Orban B. Clinical and histologic study of the surface characteristics of the gingiva. Oral Surg Oral Med Oral Pathol 1948; 1: 827‐ 841.
6. Weinmann JP, Meyer J, Mardfin D, Weiss M. Occurrence and role of glycogen in the epithelium of the alveolar mucosa and of the attached gingiva. Am J Anat 1959; 104: 381‐ 402.
7. Lozdan J, Squier CA. The histology of the muco‐ gingival junction. J Periodontal Res 1969; 4: 83‐ 93.
8. Kapur KK, Chauncey HH, Shapiro S, Shklar G. A comparative study of the enzyme histochemistry of human edentulous alveolar mucosa and gingival mucosa. Periodontics 1963; 1: 137‐ 147.
9. Tencate AR. The distribution of acid phosphatase, non‐ specific esterase and lipid in oral epithelia in man and the macaque monkey. Arch Oral Biol 1963; 8: 747‐ 753.
10. Kojović D, Pejčić A, Obradović R, Marjanović D. Parodontologija, Galaksija Niš, 2015.
11. Sheehan DC, Hrapchak BB. Staining and labelling‐ laboratory manuals. In: Sheehan DC, Hrapchak BB, editors. Theory and Practice of Histotechnology. 2nd ed. St. Louis: CV Mosby Publishers; 1980. p. 219.
12. Fasske E, Morgenroth K. Comparitive stomatoscopic and histochemical studies of the marginal gingiva in man. Parodontologie 1958; 12: 151‐ 160.
13. Bowers GM. A study of the width of the attached gingiva. J Periodontology, 1963; 34: 210-213.
14. Ainamo J, Talari A. The increase with age of the width of attached gingiva. J Periodontal Res 1976; 11: 182-188.
15. Tenenbaum H, Tenenbaum M. A clinical study of the width of the attached gingiva in the deciduous, transitional and permanent dentitions. J Clin Periodontol 1986; 13(4):270-275.
16. Talari A, Ainamo J. Orthopantomographic assessment of the width of attached gingiva. J Periodontal Res 1976; 11(4): 177-181.
17. Ainamo A, Ainamo J. The width of attached gingiva on supraerupted teeth. J Periodontal Res 1978; 13(3): 194-198.
18. Saario M, Ainamo A, Mattila K, Suomalainen K, Ainamo J. The width of radiologically-defined attached gingiva over deciduous teeth. J Clin Periodontology 1995; 22(12): 895-898.
19. Saario M, Ainamo A, Mattila K, Ainamo J. The width of radiologicallydefined attached gingiva over permanent teeth in children. J Clin Periodontology 1994; 21(10): 666-669.
20. Vincent JW, Machen JB, Levin MP. Assessment of attached gingiva using the tension test and clinical measurements J Periodontology 1976; 47(7): 412-414.
21. Bowers GM. A study of the width of attached gingiva. J Periodontol 1963; 34: 201‐ 209.
22. Jacob P, Zade RM. Width of attached gingiva in an Indian population: A descriptive study. Bangladesh J Med Sci 2009; 8: 1‐ 4.
23. Subbaiah R, Manohar B. Assessment of the width of attached gingiva in different regions of the mouth in an Indian subpopulation. J Indian Dent Assoc 2012; 6: 96‐ 98.
24. Guglielmoni P, Promsudthi A, Tatakis DN, Trombelli L. Intra‐ and inter‐ examiner reproducibility in keratinized tissue width assessment with 3 methods for mucogingival junction determination. J Periodontol 2001; 72: 134‐ 139.
25. Bernimoulin JP, Son S, Regolati B. Biometric comparison of three methods for determining the mucogingival junction. Helv Odontol Acta 1971; 15: 118‐ 120.

Primljen/ Recived on: 03.03.2107.
Revidiran/ Revised on: 21.03.2017.
Prihvaćen/ Accepted on:05.05.2017.

PRIKAZ SLUČAJA
CASE REPORT
doi:10.5937/asn1775718S

MOLARNO-INCIZIVNA HIPOMINERALIZACIJA: TERAPIJSKI IZAZOV U PEDONTOLOŠKOJ PRAKSI

MOLAR-INCISOR HYPOMINERALIZATION: THERAPEUTIC CHALLENGE TO PAEDIATRIC DENTISTRY PRACTICE

Branislava Stojković[1,2], Ljiljana Kostadinović[1,2], Marija Igić[1,2], Olivera Tričković Janjić[1,2], Simona Stojanović[1], Predrag Janošević[1,3]

[1]UNIVERZITET U NIŠU, MEDICINSKI FAKULTET U NIŠU, SRBIJA
[2] KLINIKA ZA STOMATOLOGIJU, PREVENTIVNA I DEČJA STOMATOLOGIJA, NIŠ, SRBIJA
[3] KLINIKA ZA STOMATOLOGIJU, ORTOPEDIJA VILICA, NIŠ, SRBIJA

[1] UNIVERSITY OF NIŠ, FACULTY OF MEDICINE, NIŠ, SERBIA
[2] CLINIC OF DENTISTRY, PAEDIATRIC AND PREVENTIVE DENTISTRY, NIŠ, SERBIA
[3] CLINIC OF DENTISTRY, JAW ORTHOPEDICS, NIŠ, SERBIA

Sažetak

Uvod: *Molarno-incizivna hipomineralizacija označava pojavu hipomineralizacije gleđi sistemskog porekla, koja zahvata od 1-4 prva stalna molara, a koja je često udružena sa promenama na stalnim sekutićima. Varijacije u kliničkoj manifestaciji, uz često prisutnu asimetriju, su razlog velikih varijacija u potrebama za terapijskim tretmanom ovih strukturnih gleđnih defekata.*
Prikaz slučajeva*: Prikazana su dva pacijenta sa dijagnozom molarno-incizivne hipomineralizacije. Prvi pacijent, devojčica stara 6 godina, kod koje je dijagnostikovana blaga klinička forma, se stomatologu javila odmah nakon erupcije prih stalnih molara. Rana dijagnoza i blagovremena primena preventivno-profilaktičkih mera, uz blagu kliničku formu, su razlozi sprečavanja nastanka karijesa i daljeg širenja poseruptivnog odlamanja gleđi na zahvaćenim prvim stalnim molarima. Nasuprot prvom, drugi pacijent, devojčica stara 7,5 godina, je sa dijagnozom umerene molarno-incizivne hipomineralizacije. Kod nje je došlo do pojave karijesa na zubu 46, kao i komplikacija karijesa karijesa na zubu 36, koji je ekstrahiran vrlo brzo nakon erupcije.*
Zaključak: *Rana dijagnoza i težina kliničke slike su najznačajni faktori koji utiču na terapijski ishod u zbrinjavanju molarno-incizivne hipomineralizacije.*

Ključne reči: *Molarno-incizivna hipomineralizacija, rana dijagnoza, terpijski tretman*

Corresponding author
Branislava Stojković,DDS,PhD student
Faculty of Medicine Niš,
Dr Zoran Djindjić Bld 81, Niš, Srbija
E-mail: sbranislava@gmail.com

Summary

Introduction: *Molar incisor hypomineralization indicates the appearance of enamel hypomineralization of systemic origin which occurs in 1-4 first permanent molars, which is often accompanied by changes on permanent incisors. Variations in clinical manifestation, together with commonly present asymmetry, are the reason for large variations in the needs for therapeutic treatment of these structural enamel defects.*
Case study: *Two patients with diagnosed molar incisor hypomineralization are shown. The first patient, a six-year-old girl, with diagnosed mild clinical form, visited the dentist immediately after the eruption of the first permanent molars. Early diagnosis and timly application of preventive-profilactic measures, together with mild clinical form, are the reason for prevention of dental caries and further spreading of posteruptive enamel brakdown on affected first molars. Unlike the first one, the second patient, a 7,5-year-old girl had the diagnosis of moderate molar incisor hypomineralization. She developed dental caries on tooth 46 as well as complication in dental caries on tooth 36 which was extracted soon after the eruption.*
Conclusion: *Early diagnosis and the severity of clinical appearance are the most important factors which determine therapeutic outcome of the treatment of molar incisor hypomineralization.*

Key words: *molar incisor hypomineralization, early diagnosis, therapeutic treatment*

Uvod

Upotrebu termina "molarno-incizivna hipomineralizacija" (MIH) su u stomatološku praksu uveli Weerheijm i sar. 2001. godine da bi označili pojavu hipomineralizacije gleđi sistemske prirode, koja zahvata od 1-4 prva stalna molara, a koja je često udružena sa hipomineralizovanim promenama na stalnim sekutićima [1]. Ubrzo nakon toga je istaknuto da se hipomineralizovane promene, osim na pomenutim zubima, istovremeno mogu javiti na ostalim stalnim zubima [2], zbog čega je upotreba ovog termina dovedena u pitanje. Međutim, sugerisano je da se termin MIH zadrži sve dok zvanično ne bude dokazano da promene nisu ograničene samo na prve stalne molare i sekutiće, kada će biti predložena revizija naziva ove pojave [3].

Prevalencija MIH pokazuje veliku varijabilnost i kreće se u rasponu od 2,5% do 40,2% [4,5]. Podaci o prevalenciji ovih defekata na ostalim stalnim zubima su oskudni, s obzirom da se epidemološka istraživanja najčešće rade u uzrastu kada ovi zubi još nisu nikli u ustima deteta [6]. Zvaničnih podataka o rasprostranjenosti MIH za našu zemlju nema, mada se procenjuje da iznosi oko 19,5% [7,8].

Osnovni patogenetski mehanizam MIH je poremećaj resorptivnog potencijala ameloblasta i inhibicija proteolitičkih enzima usled čega dolazi do zadržavanja gleđnih proteina, ometanja rasta kristala i maturacije gleđi [9-14]. Rezultat toga je promena u mineralnom sastavu gleđi, povećanje poroznosti, smanjenje tvrdoće i modula elastičnosti hipomineralizovane gleđi u odnosu na gleđ koja nije zahvaćena promenama, što se klinički manifestuje promenama u boji gleđi, koje variraju od beličastih do promena braon prebojenosti [15-17]. Osim toga, utvrđeno je da hipomineralizovana gleđ ima 3-15 puta veći sadržaj proteina u odnosu na normalnu gleđ.

Etilogija MIH još nije dovoljno jasna, mada je poznato da je MIH posledica sistemskog dejstva određenih faktora koji deluju u periodu mineralizacije prvih stalnih molara i inciziva, i to naročito onih koji deluju u toku prve godine života [18]. Do sada je opisan veliki broj potencijalnih faktora koji su prema vremenu delovanja podeljeni na prenatalne, perinatalne i postnatalne [19]. Nedavna istraživanja su sugerisala da je etiologija MIH multikazualna i da genetske varijacije u kombinaciji sa različitim faktorima sredine mogu igrati značajnu ulogu u nastanku ovih razvojnih gleđnih defekata [20].

Introduction

The term "Molar incisor hypomineralization" (MIH) was introduced in dental practice by Weerheijm et al. in 2001 to highlight the occurrence of enamel hypomineralization of systemic origin, that affects from 1-4 first permanent molars and is often accompanied with hypomineralized changes on permanent incisors [1]. It was soon, after that, pointed out that hypomineralized changes, except on the aforementioned teeth, can simultaneously occur in other permanent teeth [2], thus questioning the use of this term. However, it was suggested that the term MIH be kept until officially proved that these changes are not limited only to first permanent molars and incisors, when the revision of the term would be suggested [3].

The prevalence of MIH shows high variability and is in the range of 2.5% to 40.2% [4,5]. Data on the prevalence of these defects on other permanent teeth are scarce, given that epidemiological research is most often conducted at the age when these teeth have not yet sprung out in the mouth of the child [6]. There are no official data on the prevalence of MIH for our country, but it is estimated to be approximately 19.5% [7,8].

The main pathogenic mechanism of MIH is the disorder of adsorbent potential of ameloblasts and inhibition of proteolytic enzymes, causing retention of enamel proteins, interfering with crystal growth and maturation of enamel [9-14]. This results in the transformation of mineral composition of the enamel, increase in porosity, decrease in hardness and elasticity module of hypomineralized enamel compared to the non-affected enamel, which is clinically manifested by changes in enamel colour that varies from whitish to changes of brown discoloration [15-17]. Besides, it is established that hypomineralized enamel had 3-15fold higher protein content than the normal enamel.

Etiology of MIH is still not clear enough, but it is known that MIH is a consequence of systemic effects of certain factors that act in the period of mineralization of first permanent molars and incisors, especially those factors that act in the first two years of life [18]. A large number of potential factors have been described until now, and they have been divided into prenatal, perinatal and postnatal according to the time of action [19].

Klinička manifestacija MIH pokazuje veliku varijabilnost u broju zahvaćenih zuba i težini hipomineralizovanih promena. Prema težini kliničke slike, MIH je klasifikovana na blagu, umerenu i tešku formu [21]. Asimetrija je značajna karakteristika MIH i označava pojavu da je gleđ jednog prvog stalnog molara /inciziva zahvaćena više ili težom vrstom promena u odnosu na gleđ kontralateralnog prvog stalnog molara/inciziva [7,22]. Promene na prvim stalnim molarima variraju od beličastih zamućenosti do teških hipomineralizovanih promena praćenih pucanjem gleđi, često već u toku nicanja zuba, te dentin ostaje nezaštićen, pa se karijesna lezija lako razvija. Promene na sekutićima su uglavnom u vidu ograničenih zamućenja gleđi, od bele do braonkaste prebojenosti, najčešće bez prekida kontinuiteta gleđi. Zubi zahvaćeni promenama mogu biti osetljivi na nadražaje, što može otežati održavanje oralne higijene i ishranu pacijenta.

Dijagnostičke kriterijume MIH postavili su 2003. godine Weerheijm i sar.: ograničena zamućenost, posteruptivno odlamanje gleđi, atipične restauracije, ekstrakcija prvog stalnog molara zbog MIH, neiznikao zub [2]. Iako postavljanje dijagnoze ne predstavlja veći problem, u diferencijalnoj dijagnozi treba isključiti fluorozu zuba, strukturne defekte izazvane lokalnim faktorima, amelogenesis imperfecta, tetraciklinsku prebojenost, gleđnu hipoplaziju, karijes [3,23].

Budući da je veoma rano uočeno da zbrinjavanje MIH predstavlja izazov u svakodnevnom radu stomatologa i da je skopčano sa brojnim poteškoćama, velika pažnja je usmerena na pronalaženje terapijskih strategija u zbrinjavanju ovih gleđnih defekata.

Cilj ovog rada bio je da ukaže na značaj rane dijagnoze i značaj rane terapije molarno-incizivne hipomineralizacije.

Prikaz slučaja

Pacijent 1

Prvi prikazani pacijent je devojčica stara 6 godina, koja se u pratnji majke javila Službi za preventivnu i dečju stomatologiju Klinike za stomatologiju u Nišu zbog toga što je majka registrovala razliku u boji kvržica na zubu 36, sumnjajući na prisustvo karijesa na tom zubu. Kliničkim intraoralnim pregledom pacijenta, korišćenjem kriterijuma po Weerheijmu i sar. [2], dijagnostikovana je MIH, pri čemu su hipomineralizovane promene detektovane samo na zubima 36 i 46 u vidu beličasto-žućkastih promena.

Recent research has indicated that etiology of MIH is multicasual and that genetic variations can, in combination with different environmental factors, play a significant role in the occurrence of these structural enamel defects [20].

Clinical manifestation of MIH shows great variability in the number of affected teeth and severity of hypomineralized changes. According to the severity of clinical picture, MIH has been classified into mild, moderate and severe form [21]. Asymmetry is a significant characteristic of MIH and indicates that the enamel of the first permanent molar/incisor is more affected or is affected by more severe changes compared to the enamel of contralateral first permanent molar/incisor [7,22]. Changes on first permanent molars vary from whitish blur to heavy hypomineralized changes accompanied by cracking enamel, often during teething, so dentine remains unprotected and the carious lesion is easily developed. Changes on incisors are usually in the form of limited blurish enamel, from white to brownish discoloration, most often without interruption in enamel continuity. Teeth affected by changes may be sensitive to stimuli, which can make it difficult to maintain oral hygiene and diet of the patient.

Diagnostic criteria for MIH were set in 2003, by Weerheijm et al.: demarcated opacity, posteruptive enamel breakdown, atypical restauration, extracted molar due to MIH, unerupted tooth [2]. Although the diagnosis does not represent much of a problem, in differential diagnosis one should exclude dental fluorosis, structural defects caused by local factors, amelogenesis imperfecta, tetracycline discoloration, enamel hypoplasia, dental caries [3, 23].

Since it has been established very early that taking care of MIH presents a challenge in everyday work of dentist, and that it features many difficulties, a huge amount of attention is directed to treatment of these enamel defects.

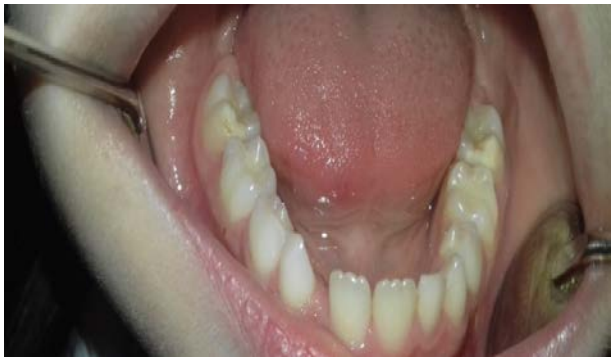
The aim of this study is to emphasize the importance of early diagnosis and early treatment of molar incisor hypomineralization.

Case study

Patient 1

The first presented patient is a 6-year-old girl, which, accompanied by her mother, visited the Department of Preventive and Paediatric Dentistry of Dental Clinic in Nis,

Na zubu 46 prisutan je blag prekid kontinuiteta gleđi. Promene nisu praćene subjektivnim tegobama, u smislu osetljivosti zuba na nadražaje. Anamnezom dobijenom od majke devojčice dobijen je podatak da kod devojčice nije dijagnostikovano nijedno hronično sistemsko oboljenje i da devojčica nije imala česte epizode febrilnih stanja tokom prvih godina života. Nakon dijagnostikovanja MIH, uklonjene su meke naslage sa zuba, izvršena lokalna fluorizacija zuba koncentrovanim fluoridima. Pacijentu je data instrukcija o tehnici, redovitosti, učestalosti i dužini pranja zuba, i preporučena upotreba preparata sa fluoridima prema važećem protokolu o primeni fluorida iz 2009. godine [24], zakazani redovni kontrolni pregledi. Godinu dana od postavljene dijagnoze MIH, na redovnom kontrolonom pregledu nije dijagnostikovana pojava karijesa na prvim stalnim molarima zahvaćenim hipomineralizovanim promenama kao i na ostalnim prisutnim stalnim i mlečnim zubima (Slika 1).



Slika 1. Zubi 36 i 46 sa beličasto-žućkastim hipomineralizovanim promenama
Figure 1. Teeth 36 and 46 with white-yellowish hypomineralizsed changes

Pacijent 2

Drugi prikazani pacijent je devojčica, stara 7,5 godina, koja se u pratnji majke javila Službi za preventivnu i dečju stomatologiju Klinike za stomatologiju u Nišu zbog sanacije karijesnih zuba. Kliničkim pregledom pacijenta, korišćenjem kriterijuma za dijagnozu po Weerheijmu i sar.[2], dijagnostikovana je MIH. Na zubima 16, 26, 46 registrovane su hipomineralizovane promene žućkasto-braonkaste prebojenosti (Slika 2). Na zubu 46 registrovane su braonkaste promene praćene prekidom kontinuiteta gleđi u distalnom delu krunice zuba, gde je dijagnostikovano prisustvo karijesa. Zubi zahvaćeni hipomineralizovanim promenama osetljivi su na termičke nadražaje. Zub 36 je ekstrahiran (Slika 3).

since the mother had registered the difference in colour of cusps on tooth 36, suspecting the presence of dental caries on that tooth. The use of clinical intraoral examination and criteria by Weerheijm et al. [2] helped to diagnose MIH with hypomineralized changes that were detected only on teeth 36 and 46 in the form of whitish-yellowish changes. The tooth 46 had slightly interrupted enamel continuity. The changes were not followed by subjective discomforts regarding sensitivity to stimuli. History, taken from the girl's mother, provided data that the girl had not been diagnosed with any chronical systemic disease, and that the girl had not had frequent episodes of febrile conditions during first years of life. After the diagnosis of MIH, soft layers were removed from the teeth, and local fluoridization was done by concentrated fluoride. The patient was instructed on the technique, frequency and duration of tooth brushing, the use of formulation with fluoride was recommended according to the current protocol on fluoride use from 2009 [24], and regular check-ups were scheduled. During control check-up, one year after diagnosing MIH, there was no diagnosis of dental caries on first permanent molars as well as on other permanent and primary teeth affected by hypomineralized changes(Figure1).

Patient 2

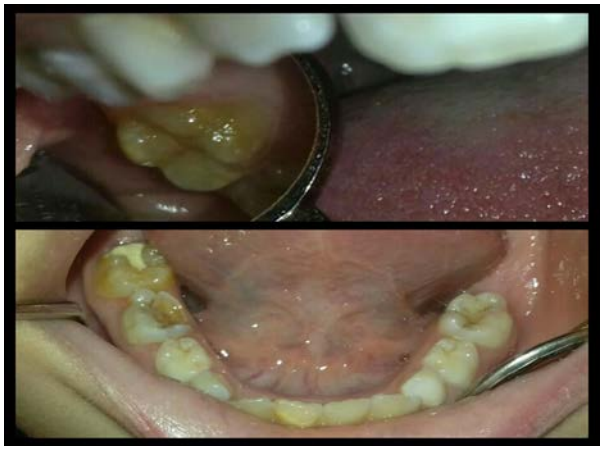
The second patient was a 7,5-year-old girl who, accompanied by her mother, visited the Department of Preventive and Paediatric Dentistry of Dental Clinic in Niš for the treatment of dental caries. Clinical examination of the patient, with the use of the diagnosing criteria according to Weerheijm et al. [2], resulted in diagnosing MIH. Teeth 16, 26, 46 had the presence of hypomineralized changes with brownish discolouration (Figure 2). The tooth 46 had hypomineralized changes with brownish discolouration followed by interrupted enamel continuity in the distal part of the tooth crown, where the presence of dental caries was diagnosed. The teeth with hipomineralized changes were sensitive to thermic stimuli. The tooth 36 was extracted (Figure 3). Teeth 11, 21 and 41 had whitish-yellowish hypomineralized changes without interrupted enamel continuity (Figure 4).

Na zubima 11, 21 i 41 registrovane su beličasto-žućkaste hipomineralizovane promene bez prekida kontinuiteta gleđi (Slika 4). Anamnezom uzetom od majke devojčice dobijen je podatak da je devojčica rođena u terminu, Carskim rezom, na rođenju normalne telesne mase. Od majke je dobijen anamnestički podatak o postojanju učestalih febrilnih stanja i čestoj upotrebi antibiotika u toku prve godine života devojčice. Nakon kliničkog pregleda pacijenta, uklonjene su meke naslage sa zuba i izvršena je sanacija karijesa na zubu 46 postavljanjem biološke paste na bazi kalcijum-hidroksida, a zub privremeno zatvoren glas-jonomer ispunom (Slika 2). Zbog hiperosetljivosti zuba 46, preparacija kaviteta II klase (okluzo-distalno) izvedena je u sprovodnoj-mandibularnoj analgeziji, ali zadovoljavajuća anelgezija nije postignuta. Izvšena je lokalna fluorizacija zuba koncentrovanim fluoridima. Pacijentu je data instrukcija o tehnici, redovitosti, učestalosti i dužini pranja zuba, a preporučena je upotreba mekane četkice za zube. Takođe, preporučena je upotreba preparata sa fluoridima prema važećem protokolu o primeni fluorida iz 2009. godine [24], zakazan nastavak terapije i redovni kontrolni pregledi.

Diskusija

Pronalaženje adekvatnog terapijskog modela i strategija u zbrinjavanju MIH bio je čest predmet istraživanja, budući da zbrinjavanje MIH predstavlja izazov i da je skopčano sa brojnim poteškoćama kao što su osetljivosti zuba na različite nadražaje, rana pojava i brza progresija karijesa, otežano postizanje lokalne analgezije, otežano postizanje adhezije restaurativnih materijala i učestalo marginalno pucanje postavljenih ispuna [10]. Osim toga, hipomineralizovane promene lokalizovane na frontalnim zubima narušavaju estetski izgled deteta, što se može negativno odraziti na njegov socijalni život. Osetljivost zuba na nadražaje je u direktnoj vezi sa težinom kliničke slike, i rezultat je subkliničke inflamacije pulpe, koja nastaje bez prethodne pojave karijesa [25]. Pojedini autori su utvrdili da oralne bakterije mogu da penetriraju kroz hipomineralizovanu gleđ u dentinske kanaliće i izazovu inflamatornu reakciju pulpe, koja može doprineti hiperosetljivosti zuba sa MIH [38]. Osetljivost zuba na nadražaje otežava održavanje oralne higijene i ishranu deteta, što olakšava akumulaciju dentalnog plaka, koji zajedno sa

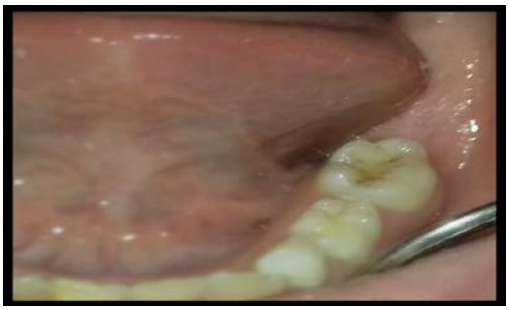
History, taken from the girl's mother, provided the data that the girl was born at term by a Caesarean section and was of normal birth weight. The mother provided information on girl's frequent febrile conditions and frequent use of antibiotics within the first year of her life. After clinical examination, soft layers were removed from the teeth and the dental caries were treated on tooth 46 by setting a biological paste containing calcium hydroxide, and the tooth was temporarily closed with glass-ionomer fillings (Figure 2). Due to hypersensitivity of the tooth 46, the preparation of class II cavity (occlusion-distal) was done in conductive-mandibular analgesia, but satisfactory analgesia was not achieved. Local fluoridization was done by concentrated fluoride. The patient was instructed on the technique, frequency and duration of tooth brushing and the use of soft toothbrush was recommended. The use of formulation with fluoride was also recommended according to the current protocol on fluoride use from 2009 [24], and further treatment and regular check-ups were scheduled.



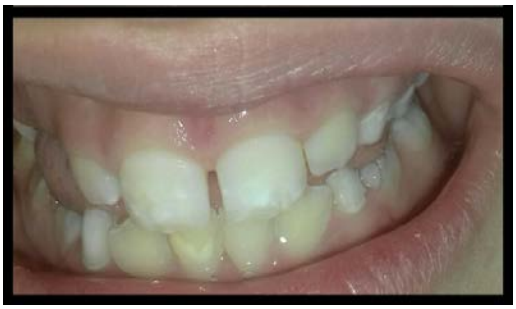
Slika 2. Hipomineralizovani prvi stalni molari 16,46
Figure 2. Hypomineralized first permanent molars 16,46

Discussion

Finding adequate therapeutic models and strategies in managing MIH was a frequent research subject since MIH treatment is a challenge and is intertwined with numerous difficulties such as teeth sensitivity to different stimuli, early occurrence and rapid progression of dental caries, difficulty in achieving local analgesia, difficulty in achieving adhesion of restorative materials



Slika 3. Ekstrahiran zuba 36 - rezultat komplikacija karijesa na zubu zahvaćenom hipomineralizovanim promenama
Figure 3. Extracted tooth 36 - the result of caries complications on the tooh with hypomineralized changes



Slika 4. Beličasto-žućkaste hipomineralizovane promene na stalnim sekutićima (11, 21, 41)
Figure 4. White-yellowish hypomineralized changes on permanent incisors 11,21,41

oslabljenom strukturom zuba doprinosi lakšoj pojavi i bržoj progresiji karijesa. Iako je utvrđena signifikantna povezanost između MIH i pojave karijesa, neke studije su potvrdile da su neophodne dobro dizajnirane studije koje bi ovu povezanost i potvrdile [36].

Postizanje lokalne analgezije kod ove grupe pacijenata je otežano, mada tačan mehanizam tome nije poznat. Imunocito-hemijska istraživanja Rodd i sar. su pokazala da hipomineralizovani prvi stalni molari pokazuju promene u inervaciji pulpe, vaskularizaciji, akumulaciji imunoloških ćelija koje su indikativne za inflamatorni odgovor [26]. Nalazi ovih autora bi delimično objasnili zašto postizanje lokalne analgezije kod ovih pacijenata može biti otežano. Neki autori su mišljenja da određeni broj pacijenata sa MIH zahteva zbrinjavanje zuba u opštoj anesteziji [27]. Kod pacijenata sa MIH, osetljivost zuba, rana pojava karijesa i otežano postizanje analgezije su glavni uzroci straha od stomatoloških intervencija i njihovo izbegavanje.

and frequent marginal cracks on set fillings [10]. In addition, hypomineralized changes localized on the front teeth disturb aesthetic appearance of the child and may negatively affect his/her social life.Tooth sensitivity to stimuli is directly connected to the severity of clinical picture, and it is the result of subclinical inflammation of the pulp, which appears without the occurrence of dental caries [25]. Some authors have found that oral bacteria may penetrate the hypomineralized enamel into the dentinal tubules and cause inflammatory reactions in the pulp, which can contribute to hypersensitivity of teeth with MIH [38]. Tooth sensitivity to stimuli hinders maintenance of oral hygiene and diet of a child, thus facilitating accumulation of dental plaque, which, together with weakened tooth structure, contributes to easier occurrence and faster progression of dental caries. Although a significant association between MIH and caries was found, some studies confirmed the need for well-designed studies to provide evidence of this association [36].

Achieving local analgesia in this group of patients is difficult, although the exact mechanism for this is not known. Immunocytochemical investigations of Rodd et al. have shown that hipomineralized first permanent molars demonstrated changes in pulpal innervation, vascularity and accumulation of immune cells which are indicative of an inflammatory response [26]. The findings of these authors could partly explain why the achievement of local analgesia in these patients may be difficult. Some authors are of the opinion that a certain number of patients with MIH require dental care under general anaesthesia [27]. In patients with MIH, tooth sensitivity, early dental caries, and difficulty in achieving analgesia are the main causes of fear of dental procedures and their avoidance. The need for therapeutic treatment shows great variations and is ranked from prevalence, through restauration to extraction [3], whereby in some cases there is a need to satisfy functional and other aesthetic demands. The choice of therapeutic treatment depends on severity of clinical picture, number of affected teeth, degree of child's cooperativeness, age when consulting the dentist, social-economic status, and the need for orthodontic treatment [3]. There is no universal guide, but numerous therapeutic approaches and strategies in treating MIH have been described up to now.

Potrebe za terapijskim tretmanom pokazuju velike varijacije i rangirane su od prevencije, preko restauracije do esktrakcije [3], pri čemu u pojedinim slučajevima treba zadovoljiti funkcionalne, a u drugim estetske zahteve. Izbor terapijskog tretmana zavisi od težine kliničke slike, broja zahvaćenih zuba, stepena saradljivosti deteta, uzrasta kada se javilo stomatologu, socio-ekonomskog statusa, potrebe za ortodontskim tretmanom[3]. Univerzalni vodič ne postoji, ali je do sada opisan veći broj terapijskih pristupa i strategija u zbrinjavanju MIH. Terapijski pristup koji su predložili William i sar. [10] 2006. godine obuhvata identifikaciju faktora rizika koji dovode do nastanka MIH, ranu dijagnostiku, remineralizaciju i desenzitizaciju, prevenciju karijesa i posteruptivnog odlamanja gleđi, restauracijiu i ekstrakciju i održavanje postignutih rezultata. Mathu-Muju i Wright [28] su iste godine predložili terapijski pristup baziran na težini kliničke slike, dok su Lygidakis i sar. [3] 2010. godine predložili pristup koji je baziran na tipu denticije i težini kliničke slike.

Identifikacija deteta koje je u riziku od pojave MIH bi bila od velikog značaja, jer bi omogućila ranu dijagnozu MIH, a time i blagovremenu primenu preventivnih mera. Međutim, nju otežava činjenica da etiološki faktori koji dovode do pojave MIH još uvek nisu jasno determinisani. Literaturni podaci ukazuju da se sa nastankom MIH najčešće povezuju hipoksična stanja u zadnjem mesecu trudnoće, prevremeno rođenja deca, učestale epizode febrilnih stanja i česta upotreba antibiotika u najranijem detinjstvu, astma, deficit vitamina D, poremećaji metabolizma kalcijuma i fosfata, zbog čega se pobrojani faktori mogu koristiti za identifikaciju osoba koje su u riziku za pojavu MIH. Neophodne su dalje prospektivne studije koje će rasvetliti etiologiju ove pojave i time omogućiti ranu identifikaciju osoba koje su u riziku od pojave MIH.

Rana dijagnostika MIH bi omogućila primenu odgovarajućih mera koje imaju za cilj da spreče rani gubitak tvrdih zubnih tkiva i pojavu karijesa, kao i da ublaže osetljivost zuba na različite nadražaje [10]. Istovremeno, rana dijagnostika bi omogućila rani tretman zuba, čime bi se smanjila potreba za retretmanom, a mogućnost pojave komplikacija koje mogu uzrokovati ekstrakciju zuba bi se svela na minimum. Za ranu dijagnostiku MIH neophodni su redovni kontrolni pregledi, naročito u periodu kada se očekuje erupcija prvih stalnih molara i sekutića, što odgovara periodu od 5. do 7. godine života.

Therapeutic approach suggested by William et al. [10] in 2006 includes identification of risk factors which lead to the occurrence of MIH, early diagnosis, remineralization and desensitization, prevention of dental caries and post-eruptive breakage of enamel, restauration and extraction, as well as maintenance of achieved results. The same year, Mathu-Muju and Wright [28] suggested therapeutic approach based on the severity of clinical picture, while Lygidakis et al. [3] in 2010 suggested the approach based on the type of dentition and severity of clinical picture.

Identification of the child that is at risk of MIH would be extremely important since it would enable early diagnosis of MIH and, at the same time, timely implementation of preventive measures. However, this is aggravated by the fact that etiologic factors which lead to MIH occurrence are still not clearly determined. Literature data indicate that formation of MIH is most often connected with hypoxic conditions in the last month of pregnancy, preterm birth, frequent febrile conditions and frequent use of antibiotics in the earliest childhood, asthma, deficiency of vitamin D, metabolic disorders of calcium and phosphate which is why the listed factors can be used to identify people who are at risk of MIH occurrence. There is a need for prospective studies to elucidate the aetiology of this phenomenon, and thereby enable early identification of people at risk of developing MIH.

Early diagnostics of MIH would enable the application of appropriate measures that are intended to prevent the early loss of dental hard tissues and dental caries, as well as to alleviate tooth sensitivity to different stimuli [10]. At the same time, early diagnostics would enable early treatment of teeth which would decrease the need for retreatment thus reducing the possibility of complications leading to teeth extraction to minimum. Early diagnostics of MIH demands regular check-ups, especially in the period when eruption of first permanent molars and incisors is expected, which is usually in the age of 5 to 7 years. Additionally, parents' knowledge on this type of defects would be of great help. This study describes the case of a girl with early diagnosed mild form of MIH, immediately after tooth eruption. In these patients it is recommended that oral hygiene, is performed by using a soft brush, it is advisable to use local low-concentrated fluoride, to apply formulations based on casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), undergo professional

Osim toga, od velike pomoći bi bila i informisanost roditelja o ovoj vrsti defekata. U ovom radu opisan je slučaj devojčice kod koje je blaga forma MIH rano dijagnostikovana, neposredno nakon erupcije zuba. Kod ovakvih pacijenata preporuka je da se oralna higijena obavlja upotrebom mekane četkice za zube, savetuje se lokalna upotreba niskokoncentorvanih fluorida, aplikovanje preparata na bazi kazein fosfopeptida-amorfnog kalcijum fosfata (CPP-ACP), profesionalna aplikacija visoko koncentrovanih fluorida u vidu lakova, zalivanje fisura [3,10]. Preporuka je da se fisure najpre zaliju GJC zalivačima, koji će kasnije biti zamenjeni kompozitnim. Zbog karakteristika hipomineralizovanih promena i loše adhezije materijala, neophodno je redovno kontrolisati zalivače i ukoliko dođe do pucanja ili ispadanja, postupak zalivanja fisura treba ponoviti. U opisanom prvom slučaju dat je savet o održavanju higijene i izvršena je primena preparata fluorida prema važećem protokolu iz 2009. godine [24]. Pacijent je redovno kontrolisan, motivisan i remotivisan, te na redovnom kontrolnom pregledu, godinu dana kasnije, na zubima zahvaćenim promenama nije dijagnostikovan karijes i širenje posteruptivnog odlamanja gleđi.

Međutim, rana dijagnoza MIH može biti od značaja kada se pacijent stomatologu javi na vreme, pre nego se javi potreba za klasičnim konzervativnim tretmanom, što u praksi nažalost nije slučaj. Osim toga, rana dijagnoza je od značaja samo kod lakših formi MIH, jer u umerenim i teškim kliničkim formama, već u toku erupcije zuba, dolazi do značajnog posteruptivnog odlamanja gleđi i pojave karijesa, što zahteva konzervativo zbrinjavanje, a često i ekstrakciju zuba. U drugom opisanom slučaju, devojčica se stomatologu javila kada su se komplikacije već javile, u smislu pojave karijesa na zubu 46, koji zahteva klasičan restaurativni tretman nakon terapije dubokog karijesa. Zub 36 je rano ekstrahiran, zbog, po rečima majke, velike destrukcije krunice zuba usled karijesa i rane pojave komplikacija karijesa na tom zubu.

Nedavno istraživanje je pokazalo da postoji veliki disparitet među kliničarima u pogledu načina zbrinjavanja zuba sa MIH [39]. Zbrinjavanje prvih stalnih molara zahvaćenih hipomineralizovanim promenama može predstavljati veliki problem zbog otežanog postizanja adhezije restaurativnih materijala i učestalog marginalnog pucanja postavljenih ispuna, zbog čega se često javlja potreba za retretmanom [29].

application of highly concentrated fluoride in the form of varnish, conduct sealing of fissures [3.10].

It is recommended that fissures are initially sealed with GIC sealants, which will later be replaced by composit-resins ones. Because of characteristics of hypo-mineralized changes and poor material adhesion, it is necessary to control sealants, and the procedure of fissure-sealing must be repeated, in case of breakage or failure. In the first described case, the advice on maintaining hygiene is given, and the application of fluoride formulations, according to the Protocol of 2009, was conducted [24]. The patient had regular check-ups, was motivated and re-motivated and regular check-up after one year showed that teeth affected by changes had no dental caries or expansion of post-eruptive enamel breakage.

However, early diagnosis of MIH may be significant when the patient timely addresses the dentist before there is a need for classic, conservative treatment, which, unfortunately, is not usually the case. Apart from that, early diagnosis is significant only in mild forms of MIH, since in moderate and severe clinical form during eruption, there is significant posteruptive enamel breakdown and appearance of dental caries, which demands conservative treatment or often tooth extraction. The other case describes a girl who came to the dentist after the complications had appeared in the sense of dental caries on tooth 46 which calls for classic restorative treatment after the therapy of deep dental caries. Tooth 36 was early extracted because of, according to mother's words, huge destruction on tooth's crown due to dental caries and early occurrence of dental caries complications.

Recent investigation shows that there is a wide disparity between clinicians' views on how MIH-affected teeth should be treated [39].Treatment of first permanent molars affected by hypomineralized changes may present a huge problem due to difficulty of achieving adhesion of restorative materials and frequent marginal breakage of set fillings, which frequently results in need for retreatment [29].

Due to the great significance of the first permanent molars, it is recommended that these teeth are treated in a conservative manner whenever possible. Atypical forms of cavities are characteristic for MIH and two approaches are presented regarding cavity preparation: one that involves removing only the porous enamel, and another, more radical, which involves the healthy tissue [3,10].

Zbog višestrukog značaja prvih stalnih molara, preporuka je da se ovi zubi konzervativno zbrinu kada god je to moguće. Za MIH su karakteristični kaviteti atipičnih oblika, a opisana su dva pristupa u preparaciji kaviteta: jedan koji podrazumeva uklanjanje samo porozne gleđi i drugi koji je radikalniji i koji podrazumeva uklanjanje celokupne defektne gleđi do zdravog tkiva [3,10]. Materijal izbora za restauraciju su materijali na bazi kompozitnih smola, kod kojih su preporučivani različiti pretretmani, sa ciljem da se poveća adhezija materijala za hipomineralizovanu gleđ [28,30,31]. Upotreba glas jonomer cemenata ograničena je samo na privremeno zatvaranje kaviteta i u situacijama kad je kontrolisanje vlage otežano, dok upotreba amalgama, zbog njihove neadhezivnosti, nije indikovana. U težim slučajevima, za zbrinjavanje hipomineralizovanih promena na prvim stalnim molarima mogu se uspešno koristiti i metalne krunice [32,33], a opisana je mogućnost protetske rehabilitacije zuba, mada je njihova primena u dečjem uzrastu prilično ograničena.

Ekstrakcija prvih stalnih molara je često terapija izbora u zbrinjavanju MIH, naročito u težim slučajevima. Međutim, konačnu odluku o ekstrakciji ovih zuba treba doneti u konsultaciji sa ortodontom, te je multidisciplinarni pristup u zbrinjavanju MIH od velikog značaja.

Promene na stalnim sekutićima narušavaju estetki izgled pacijenta, a njihovo zbrinjavanje zavisi od težine kliničke slike. Prilikom izbora načina zbrinjavanja hipomineralizovanih promena na sekutičima treba imati u vidu da žućkasto-braonkasti defekti zahvataju celu debljinu gleđi, dok su beličasti defekti manje porozni i variraju u debljini [34]. U lakšim slučajevima, kada estetetika nije mnogo ugrožena i kada nije došlo do prekida kontinuiteta gleđi, preporučuje se upotreba kazein fosfopeptida-amorfnog kalcijum fosfata i visoko koncentrovanih fluorida u cilju remineralizacije i smanjenja osetljivosti zuba na nadražaje, kao i tretman mikroabrazijom i izbeljivanjem [10]. Ukoliko je došlo do prekida kontinuiteta gleđi, ili zbog narušenog estetskog izgleda deteta, hipomineralizovanu promenu je moguće zbrinuti klasičnim konzervativnim tretmanom kompozitnim smolama ili vinirima, mada njihova upotreba u dečjem uzrastu može biti ograničena. Takođe je istaknuta i upotreba infiltracije smolom u zbrinjavanju ovih defekata. Iako je utvrđeno da infiltrati smolom mogu da prodru u MIH lezije i da utiču na obim lezije, promene u tvrdoći, rezultati u tom smislu su nepredvidivi [35].

The material of choice for restoration is resin-composite materials, in which various pre-treatments have been recommended in order to increase their adhesion to hypomineralized enamel [28, 30, 31]. The use of glass ionomer cements is limited to the temporary closure of the cavity and in situations where it is difficult to control the moisture, while the use of amalgams due to their lack of adhesion is not indicated. Metal crowns can successfully be used in severe cases of treating hypomineralized changes on the first permanent molars [32, 33], and there is a possibility of prosthetic rehabilitation of teeth, although their use in children is rather limited.

Extraction of first permanent molars is a commonly chosen therapy when treating MIH, especially in severe cases. However, the final decision on the extraction of these teeth should be made in consultation with the orthodontist, and a multidisciplinary approach to their care is of great importance.

Changes on permanent incisors ruin aesthetic appearance of the patient and their treatment depends on severity of clinical picture, but it should be borne in mind that the yellowish-brownish defects occupy the entire thickness of the enamel, while whitish defects are less porous and vary in thickness [34]. In mild cases, when aesthetics is not much affected and when there has been no interruption in continuity of the enamel, the use of casein phosphopeptide-amorphous calcium phosphate and highly concentrated fluoride is recommended in order to remineralize and reduce tooth sensitivity to stimuli, as well as the treatment of micro abrasion and bleaching [10]. If there is an interruption in continuity of the enamel, or because of poor aesthetic appearance of the child, a hypomineralized change can be treated with classic conservative treatment with composite resins or veneers although their use in children may be limited. The use of resin infiltration is also highlighted in the treatment of these defects. Although it was determined that resin infiltrates can penetrate into the MIH lesions and may affect the extent of lesions, changes in hardness, the results in this regard are unpredictable [35]. In addition to aesthetic interference, hypomineralized defects on incisors cause difficulty in adhesion of orthodontic brackets, and if there is a need for setting fixed orthodontic apparatus, a pre-treatment of hypomineralized enamel is recommended in order to increase the adhesiveness of hypomineralized enamel.

Osim estetskih smetnji, hipomineralizovani defekti na sekutićima izazivaju i poteškoće u adheziji ortodontskih bravica, te se, ukoliko postoji potreba za postavljanjem fiksnog ortodontskog aparata, preporučuje pretretman hipomineralizovane gleđi u cilju povećanja adhezivnosti za hipomineralizovanu gleđ.

Zaključak

Zbrinjavanje molarno-incizivne hipomineralizacije i dalje predstavlja veliki izazov u svakodnevnoj pedontološkoj praksi. Izbor terapijskog tretmana i terapijski ishod zbrinjavanja MIH su uslovljeni velikim brojem faktora, a može se smatrati da rana dijagnoza i težina kliničke slike u tom smislu igraju presudnu ulogu. Rana dijagnoza MIH bi omogućila blagovremenu primenu mera koje imaju za cilj da spreče rani gubitak tvrdih zubnih tkiva i pojavu karijesa, da ublaže osetljivost zuba na nadražaje, a istovrmeno bi omogućila rani tretman, čime bi se smanjila potreba za retretmanom i mogućnost pojava komplikacija koje mogu biti uzrok ekstrakciji zuba. Za ranu dijagnostiku MIH neophodni su redovni kontrolni pregledi, naročito u periodu kada se očekuje erupcija prvih stalnih molara i sekutića, a od velike pomoći bi bila i informisanost roditelja o ovoj vrsti defekata.

Conclusion

Treatment of molar-incisor hypomineralization still presents a big challenge in everyday practice of paediatric dentistry. The choice of therapeutic treatment and the results of treating MIH are conditioned by numerous factors, and it can be considered that early diagnosis and severity of clinical picture in that sense play a crucial role. Early diagnosis of MIH would enable timely implementation of measures in order to prevent early loss of hard tooth tissues and occurrence of dental caries, reduce sensitivity to stimuli and, at the same time, enable early treatment thus reducing the need for re-treatment and reducing the possibility of complications that can cause extraction of teeth. Early diagnosis of MIH calls for regular check-ups, especially in the period when eruption of first permanent molars and incisors is expected and parents' knowledge on this type of defects would be of great help.

LITERATURA / REFERENCES

1 Weerheijm KL, Jälevik BA, Alaluusua S. Molar-incisor hypomineralisation. Caries Res 2001; 35(5):390-1.
2. Weerheijm KL, Duggal M, Mejare I, Papagiannoulis L, Koch G, Martens LC, et al. Judgement criteria for molar incisor hypomineralization (MIH) in epidemiologic studies: a summary of the European meeting on MIH held in Athens, 2003. Eur J Paediatr Dent 2003;4:110-113.
3. Lygidakis NA, Wong F, Jälevik B, et al. Best clinical practice guidance for clinicians dealing with children presenting with molar–incisor–hypomineralisation (MIH): an EAPD policy document. Eur Arch Paediatr Dent. 2010;11(2):75–81.
4. Cho SY, KI Y, Chu V. Molar incisor hypomineralization in Hong Kong Chinese children. International Journal of Paediatric Dentistry 2008; 18(5):348-352.
5. Soviero V et al. Prevalence and distribution of demarcated opacities and their sequelae in permanent 1st molars and incisors in 7 to 13-year-old Brazilian children. Acta Odontologica Scandinavica 2009; 67(3): 170-175.
6. Schmalfuss A et al. Canines are affected in 16-year-olds with molar–incisor hypomineralisation (MIH): an epidemiological study based on the tromsø study:" fit futures". European archives of paediatric dentistry 2016; 17(2): 107-113.
7. Ivanović M et al. Treatment options for hypomineralized first permanent molars and incisors. Stomatološki glasnik Srbije 2006; 53(3):174-180.
8. Ivanović M, Živojinović V, Vučetić M. Molar Incisor Hypomineralisation, 10th Congres of the Bass, Belgrade. Balcan Journal of Stomatology 2005; 9:40.
9. Farah RA, Monk BC, Swain MV, Drummond BK. Protein content of molar–incisor hypomineralisation enamel. J Dent 2010;38:591–6.
10. William V, Messer LB, Burrow MF. Molar incisor hypomineralization: review and recommendations for clinical management. Pediatric dentistry 2006; 28(3): 224-232.
11. Wright JT, Hall K, Yamauchi M. The protein com¬position of normal and developmentally defective enamel. Ciba Found Symp 1997;205:85-99.
12. Suga S. Enamel hypomineralization viewed from the pattern of progressive mineralization of human and monkey developing enamel. Adv Dent Res 1989;3:188-198.
13. Sato K, Hattori M, Aoba T. Disturbed enamel min¬eralization in a rat incisor model. Adv Dent Res 1996;10:216-224.
14. Robinson C, Brookes SJ, Bonass WA, Shore RC. Enamel maturation. Ciba Found Symp 1997;205:156-174.
15. Martinović B et al. Analysis of the mineral composition of hypomineralized first permanent molars. Vojnosanitetski pregled 2015; 72(10): 864-869.
16. Fagrell TG et al. Chemical, mechanical and morphological properties of hypomineralized enamel of permanent first molars. Acta Odontologica Scandinavica 2010; 68(4): 215-222.
17. Mahoney EK. Mechanical properties and microstruc¬ture of hypomineralized enamel of permanent teeth. Biomaterials 2004;25:5091-5100.
18. Reid D J, Dean M C. Variation in modern human enamel formation times. J Hum Evol 2006; 50: 329-46
19. Lygidakis NA, Dimou G, Marinou D. Molar-incisor-hypomineralisation (MIH). A retrospective clinical study in Greek children. II. Possible medical aetiological factors. European Archives of Paediatric Dentistry 2008; 9(4): 207-217.
20. Jeremias F et al. Genes expressed in dental enamel development are associated with molar-incisor hypomineralization. Archives of oral biology 2013; 58(10):1434-1442.
21. Leppäniemi A, Lukinmaa PL, Alaluusua A. Nonfluoride hypomineralizations in the permanent first molars and their impact on the treatment need. Caries Res 2001;35:36-40.
22. Ivanović M et al. Hipomineralizacija na prvim stalnim molarima i incizivima. Srpski arhiv za celokupno lekarstvo 2007; 135 (7-8): 412-477.
23. Mast P et al. Understanding MIH: definition, epidemiology, differential diagnosis and new treatment guidelines. Eur J Paediatr Dent 2013; 14 (3): 204-8.
24. Ivanović M i sar. Protokol o primeni fluorida. Beograd: Stomatološki fakultet Univerziteta u Beogradu; 2009.
25. Jälevik, B et al. The prevalence of demarcated opacities in permanent first molars in a group of Swedish children. Acta Odontologica Scandinavica 2001; 59(5): 255-260.
26. Rodd HD, Boissonade FM, Day PF. Pulpal status of hypomineralized permanent molars. Pediatric dentistry 2007; 29(6): 514-520.
27. Jälevik B, Klingberg G. Treatment outcomes and dental anxiety in 18-year-olds with MIH, comparisons with healthy controls–a longitudinal study. International journal of paediatric dentistry 2012; 22(2): 85-91.
28. Jälevik B, Klingberg GA. Dental treatment, dental fear, and behaviour management problems in children with severe enamel hypomineralization of their first permanent molars. Int J Pediatr Dent 2002;12:24-32
29. Mathu-Muju K, Wright JT. Diagnosis and treatment of molar incisor hypomineralisation. Compend Contin Educ Dent 2006; 27(11): 604-10.
30. N.A. Lygidakis, G. Dimou, E. Stamataki. Retention of fissure sealants using two different methods of application in teeth with hypomineralised molars (MIH): a 4 year clinical study, Eur. Arch. Paediatr. Dent. 10 (2009) 223–226.
31. N.A. Lygidakis, A. Chaliasou, G. Siounas. Evaluation of composite restorations in hypomineralised permanent molars: a four year

clinical study, Eur. J. Paediatr. Dent. 4 (2003) 143–148.
32. Mejare I, Bergman E, Grindefjord M. Hypomineralized molars and incisors of unknown origin: treatment outcome at age 18 years. Int J Paediatr Dent 2005; 15: 20–28.
33. Zagdwon AM, Fayle SA, Pollard MA. A prospective clinical trial comparing preformed metal crowns and cast restorations for defective first permanent molars. Eur J Paediatr Dent 2003; 4 : 138–142.
34. Jalevik B, Noren J G. Enamel hypomineralisation of permanent first molars: a morphological study and survey of possible aetiological factors. Int J Paediatr Dent 2000; 10: 278-289.
35. Crombie F, Manton, D, Palamara J, Reynolds E.Resin infiltration of developmentally hypomineralised enamel. International journal of paediatric dentistry 2014; 24(1), 51-55.
36. Americano GCA et al. A systematic review on the association between molar incisor hypomineralization and dental caries. International Journal of Paediatric Dentistry 2017; 27(1):11-21.
37. Silva MJ et al. Etiology of molar incisor hypomineralization–A systematic review. Community dentistry and oral epidemiology 2016; 44(4): 342-353.
38. Fagrell TG., et al. Bacterial invasion of dentinal tubules beneath apparently intact but hypomineralized enamel in molar teeth with molar incisor hypomineralization. International Journal of Paediatric Dentistry 2008; 18.5: 333-340.
39. Kopperud SE, Pedersen CG, Espelid, I. Treatment decisions on Molar-Incisor Hypomineralization (MIH) by Norwegian dentists–a questionnaire study. BMC Oral Health 2016; 17(1): 3.

Primljen/ Recived on: 23.01.2017.
Revidiran/ Revised on: 16.02.107.
Prihvaćen/ Accepted on: 28.2.2017.

INFORMATIVNI RAD
INFORMATIVE ARTICLE
doi:10.5937/asn1775730D

PROTEZNI STOMATITIS – ETIOPATOGENEZA I TERAPIJSKI PRISTUP

DENTURE STOMATITIS: ETHIOPATHOGENESIS AND THERAPEUTIC APPROACH

Milica Č. Đorđević[1], Radmila R. Obradović[2], Milan N. Miljković[3], Marija R. Milošević[1], Miona Lj. Sekulić[1]

[1]UNIVERZITET U KRAGUJEVCU, SRBIJA, FAKULTET MEDICINSKIH NAUKA, KATEDRA ZA STOMATOLOGIJU
[2]ODELJENJE ZA ORALNU MEDICINU I PARODONTOLOGIJU, KLINIKA ZA STOMATOLOGIJU, MEDICINSKI FAKULTET, UNIVERZITET U NIŠU, SRBIJA
[3]MEDICINSKI FAKULTET, UNIVERZITET U NIŠU, STUDENT DOKTORSKIH STUDIJA, SRBIJA

[1]UNIVERSITY OF KRAGUJEVAC, SERBIA, FACULTY OF MEDICAL SCIENCES, DEPARTMENT OF DENTISTRY
[2]DEPARTMENT OF ORAL MEDICINE AND PERIODONTOLOGY, CLINIC OF DENTISTRY, FACULTY OF MEDICINE, UNIVERSITY OF NIŠ, SERBIA
[3]UNIVERSITY OF NIS, FACULTY OF MEDICINE, PHD STUDIES, SERBIA

Sažetak

Uvod: *Protezni stomatitis je najčešća zapaljenska reakcija koja se javlja kod osoba koje nose zubne proteze, sa najčešćom lokalizacijom na palatinalnoj sluzokoži. Etiopatogenza zapaljenja je multifaktorijalna i kompleksna. Infekcija gljivicama iz roda Candida, prevashodno Candida albicans, loša oralna higijena i dugotrajno nošenje proteze su najznačajniji etiološki faktori. Razvoju zapaljenske reakcije mogu doprineti i neki opšti činioci kao što je pušenje, upotreba lekova i sistemske bolesti, poput dijabetesa melitusa. Kako je zapaljenje najčešće bez subjektivnih simptoma, a ima veliku prevalenciju među nosiocima zubnih proteza, poželjno je pacijentima zakazivati redovne preglede kako bi se rano postavila dijagnoza i sprovela adekvatna terapija.*
Zaključak. *U radu je dat prikaz etiopatogeneze proteznog stomatitisa i najčešće terapijske procedure koje se sprovode pri njegovom lečenju.*

Ključne reči: *protezni stomatitis, Candida albicans, oralna higijena, zubne proteze*

Corresponding author:
Milica Đorđević,DDS, PhD student
Department of dentistry, Faculty of medical sciences,
University of Kragujevac
Adress: 1/15, Sremska street, 18000 Niš
Phone:+381645670127
E-mail:micamonro@gmail.com

Abstract

Introduction: *Denture stomatitis is the most common inflammatory reaction that occurs in people who wear dentures, localized mostly in the palatal mucosa. Etiophatogenesis of inflammation is multifactorial and complex. Infection by yeast of the genus Candida, mainly Candida albicans, poor oral hygiene and long-term wearing of dentures are the most important etiological factors. Factors that may contribute to the development of inflammatory reactions are some general factors, such as smoking, use of different drugs, and systemic diseases such as diabetes mellitus. As the inflammation usually goes without any symptoms and has high prevalence among denture wearers, it is desirable for patients to schedule regular examinations to obtain diagnosis early and to receive adequate therapy.*
Conclusion. *The manuscript presents the etiopathogenesis of denture stomatitis and usual therapeutic procedures that are carried out during treatment.*

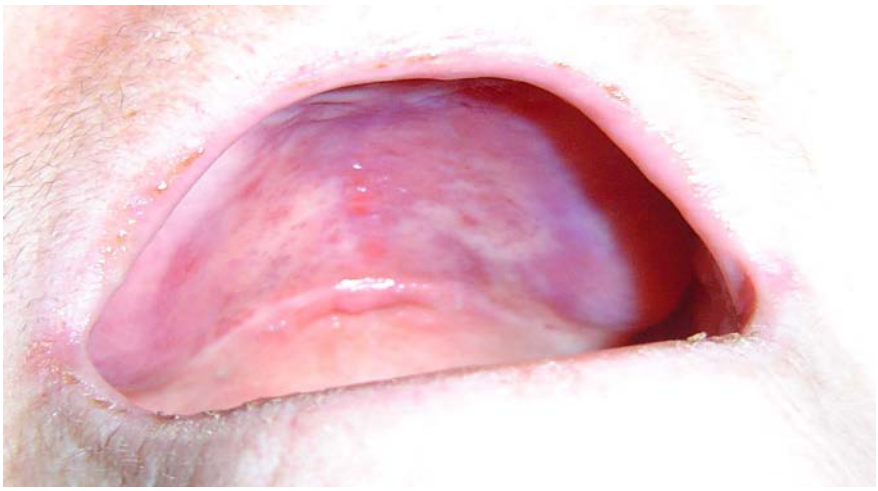
Key words: *denture stomatitis, Candida albicans, oral hygiene, dental prostheses*

Uvod

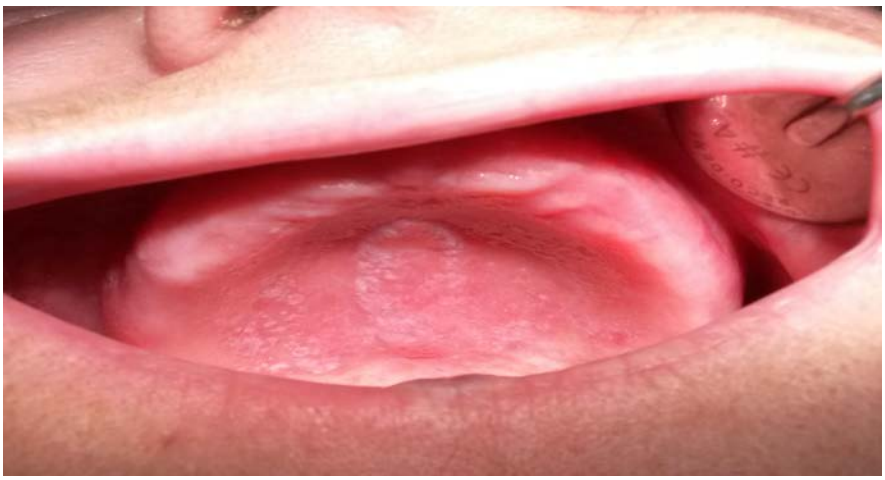
Protezni stomatitis predstavlja zapalje-nsku reakciju koja se sreće kod zdravih nosioca mobilnih zubnih proteza [1]. Najčešće se javlja na palatinalnoj i na sluzokoži alveolarnog grebena, koja ostvaruje direktan kontakt sa bazom zubne proteze [2,3]. Prevalencija se kreće u rasponu od 15% do 75%, dok je incidencija značajno veća kod starijih osoba ženskog pola [4]. Etiologija ovog zapaljenja je multifaktorijalna, a potencijalni uzročnici su: infekcija gljivicama iz roda *Candida*, bakterijske infekcije, loša oralna, loša higijena zubnih proteza, hronična iritacija sluzokože zbog slabe retencije proteze, neprekidno nošenje proteze, alergijske reakcije na materijale koji se koriste za izradu zubnih proteza, imunološki faktori, korišćenje lekova i neka sistemska oboljenja [1,5]. Još uvek se gljivice iz roda *Candida*, prvenstveno *Candida albicans*, najčešće dovode u vezu sa nastankom ovog zapaljenja [1]. Brojne studije pokazuju da dve trećine pacijenata koji nose zubnu protezu pate od ovog oblika zapaljenja [6-9]. Bez obzira na njegovu učestalost, protezni stomatitis je u najvećem broju slučajeva asimptomatski; mali broj pacijenata oseća bol, peckanje i žarenje, a same promene se primarno dijagnostikuju prilikom pregleda, kada se može primetiti eritem ili edem sluzokože koja je u kontaktu sa protezom [10]. Postoji više klasifikacija proteznog stomatitisa, ali je najrelevantnija klasifikacija koju je predložio Newton 1962. godine bazirana isključivo na kliničkim kriterijumima. Prema ovoj klasifikaciji razlikuju se tri oblika proteznog stomatitisa: 1. lokalizovano zapaljenje ili tačkasta hiperemija sluzokože (obično uzrokovana traumom) (slika 1); 2. eritematozni ili generalizovani oblik, koji obuhvata celu ili deo sluzokože koja je pokrivena protezom (slika 2); 3. granularni oblik (inflamatorna papilarna hiperplazija), koji obuhvata središnji deo tvrdog nepca i alveolarni greben (slika 3) [11].

Introduction

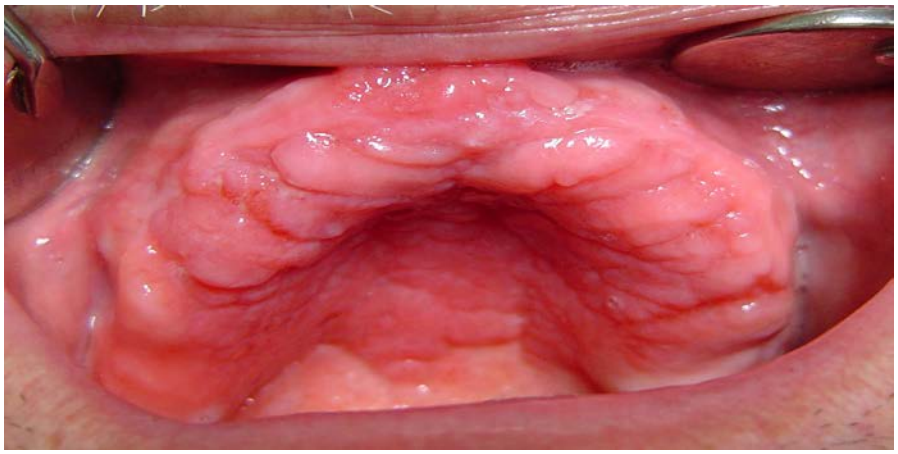
Denture stomatitis is an inflammatory reaction that occurs in healthy patients wearing dentures [1]. It is most common in the palatal mucosa and the alveolar ridge, which makes direct contact with the base of dental prostheses [2,3]. Research shows that the prevalence of denture stomatitis ranges from 15% to 75%, while the incidence is significantly higher in women and in the elderly [4]. The etiology of this inflammation is multifactorial, and the potential causes include: *Candida* yeast infections, bacterial infections, poor oral hygiene and denture hygiene, chronic irritation due to poor denture retention, constant wearing of dentures, allergic reaction to materials used for making dentures, immunological factors, the use of different drugs and some systemic diseases [1,5]. Still, *Candida* yeast infections, especially *Candida albicans*, are the most frequently associated with the development of this inflammation[1]. Numerous studies have shown that two-thirds of patients who wear dentures suffer from this type of inflammation [6-9]. Despite its prevalence, denture stomatitis in most cases is asymptomatic; a small number of patients feel pain, tingling and numbness, and the changes are primarily diagnosed during the examination, when erythema or edema can be observed, affecting the areas of the mucosa which is in contact with the prosthesis [10]. There are several classifications of denture stomatitis, but the most relevant classification was proposed by Newton in 1962, being based only on clinical criteria. According to this classification, there are three types of denture stomatitis 1. Localized inflammation of the mucosa or pinpoint hyperemia which is usually caused by trauma (Figure 1); 2. Erythematous or generalized type, involving a part or the entire denture-covered mucosa (Figure 2); 3. Granular type (inflammatory papillary hyperplasia), involving the central part of the hard palate and the alveolar ridge (Figure 3) [11].



Slika 1. Tačkasta hiperemija sluzokože uzrokovana traumom pri nošenju gornje totalne proteze
Figure 1. Localized pinpoint hyperemia of the mucosa, which is caused by denture trauma.



Slika 2. Eritematozni (generalizovani oblik) proteznog stomatitisa
Figure 2. Erythematosus (generalized type) denture stomatitis.



Slika 3. Granularni oblik (inflamatorna papilarna hiperplazija) proteznog stomatitisa
Figure 3. Granular type (inflammatory papillary hyperplasia) of denture stomatitis.

Etiopatogenetski aspekti

Protezni stomatitis je u većini slučajeva posledica hronične infekcije ili mehaničke iritacije i traume. Danas se smatra da je etilogija multifaktorijalna, što znači da će se zapaljenski proces češće razviti pri sinergističkom delovanju većeg broja faktora nego pri delovanju jednog. Loša oralna higijena, patogena infekcija gljivicom iz roda *Candida* i dugotrajno nošenje proteze su dominantni udruženi etiološki faktori koji dovode do razvoja zapaljenja [12].

Samo prisustvo proteze u usnoj duplji može delovati kao katalizator koji doprinosi pojavi proteznog stomatitisa. Njeno prisustvo menja lokalnu mikrosredinu tako što smanjuje nivo pH, protok pljuvačke i mehaničko samočišćenje, delujući kao rezervoar za razvoj mikroorganizama [3]. *Candida albicans* je mikroorganizam koji je najčešće izolovan iz sluzokože nepca (89%) i/ili sa gornje proteze (78,5%) kod pacijenata sa proteznim stomatitisom [13]. Uloga *C. albicans* u razvoju zapaljenja povezana je sa činjenicom da ova gljivica ima sposobnost da kolonizuje oralnu sluzokožu i površinu proteze i da gradi agregate sa oralnim bakterijama [14]. *C. albicans* je normalni stanovnik usne duplje, a u slučaju imunodeficijencije može postati patogena kada dovodi do razvoja kandidijaze. Protezni stomatitis može biti udružen sa angularnim heilitisom, atrofičnim glositisom, akutnom pseudomembranoznom kandidijazom i hroničnom hiperplastičnom kandidijazom [15]. Pored *C. albicans*, izolovane su i druge vrste roda *Candida* koje učestvuju u nastanku proteznog stomatitisa: *C.glabrata, C.tropicalis, C.krusei, C. kefir i C.dubliniensis* [16]. Ipak, njihova uloga u razvoju ovog zapaljenskog procesa je mnogo manja. Kod pacijenata sa proteznim stomatitisom izolovane su i određene bakterijske vrste poput *stafilokoka, sreptokoka, fuzobakterija i bakteroidesa* [3].

Među faktorima koji utiču na adherenciju *Candide* i formiranje biofilma na površini akrilata, ističu se hrapavost površine proteze, hidrofobne i elekrostatske interakcije[17]. Hrapava površina akrilata, koji se najčešće koristi kao materijal za izradu proteza, promoviše povećano zadržavanje mikroorganizama i štiti ih od sila koje teže da ih uklone, pri čemu mikroorganizmi ostaju zarobljeni nepravilnom površinom proteze, čak i nakon njenog čišćenja. Poliranje proteza čini njihovu površinu glatkom i smanjuje inicijalnu adheziju i akumulaciju mikroorganizama [18].

Etiopathogenic aspects

Denture stomatitis is in most cases a consequence of chronic infection or mechanical irritation and trauma. It is considered that the etiology of denture stomatitis is multicaftorial, and it is more likely to develop due to synergy with other factors than during the action of just one of them. Poor oral hygiene, pathogenic infection caused by *Candida* and constant wearing of dentures are dominant synergistic etiological factors that lead to inflammation [12].

The presence of dentures in the mouth can act as a catalyst that contributes to denture stomatitis. Its presence change the local microenvironment by reducing the pH level, the flow of saliva and a mechanical self-cleaning, by acting, in this way, as a reservoir for the growth of microorganisms [3]. *Candida* albicans is a microorganism which is most frequently isolated from the palatal mucosa (89%) and/or the upper prosthesis (78.5%) in patients suffering from denture stomatitis [13]. The role of *C. albicans* in the development of inflammation is associated with the fact that this yeast has the ability to adhere and colonize the oral mucosa and the surface of the prosthesis and the ability to build aggregates with oral bacteria [14]. *C.albicans* is a normal inhabitant of the oral cavity, and in the case of immune deficiency can become a pathogen when it leads to the development of candidiasis. Denture stomatitis may be associated with angular heilitis, atrophic glossitis, acute pseudomembranous candidiasis and chronic hyperplastic candidiasis [15]. In addition to *C.albicans*, other species of the genus *Candida* were isolated, participating in the development of denture stomatitis: *C.glabrata, C.tropicalis, C.krusei, C. kefir, C.dubliniensis* [16]. However, their role in the development of this inflammatory process is much smaller. In patients with denture stomatitis, certain bacterial species such as *Staphylococcus, Streptococcus, Fusobacterium* and *Bacteroides* were isolated [3].

Among the factors which influence the adherence of *Candida* and the formation of biofilm on the surface of acrylate, there are roughness of the inner surface of the prosthesis, hydrophobic and electrostatic interactions [17]. The rough surface of the acrylate, which is most commonly used as a material for making dentures, promote increased retention of microorganisms and protect them from the forces that seek to remove them, in which microorganisms are

Ovo je pokazano u studiji Sesma i saradnika [19]. Starost proteze je takođe bitan faktor, jer kod dugo nošenih proteza teže se održava higijena i prisutna je sklonost ka poroznosti baze proteze, što favorizuje nastanak infekcije [20].

Uticaj pljuvačke na adheziju *C.albicans* za površinu proteze još uvek je kontraverzan. Neke studije ukazuju da pljuvačka smanjuje adheziju gljivice. Sa druge strane, pljuvačka sadrži zaštitne molekule kao što su lizozim, laktoferin, kalprotektin i imunoglobulin IgA, koji smanjuju adheziju *C.albicans* za oralne površine [3]. Smanjenje ili kompletno odsustvo pljuvačke kod osoba sa kserostomijom izaziva disbalans u normalnom mikrobiološkom sastavu, favorizujući proliferaciju bakterije *Staphylococcus* aureus koja inhibira adaptaciju komensala [21].

U prostoru između gingivalne površine baze gornje totalne proteze i sluzokože nepca usled dobrog zaptivanja smanjuje se pljuvačni protok i dinamika promene pH pljuvačke, što dovodi do stvaranja kisele sredine i povoljnih uslova za nastanak infekcije. U donjoj vilici ventilni učinak zubne proteze je slabiji tako da se menja i pH i protok pljuvačke, zbog čega je u donjoj vilici ređa pojava proteznog stomatitisa.

Sa druge strane, hidrofobnost površine proteze utiče na adheziju *C.albicans* tako što hidrofobne površine smanjuju ćelijsku adheziju [22]. Što se tiče elektrostatske interakcije, gljivice čija površina ima pozitivno naelektrisanje su mnogo adhezivnije za razliku od odbojnih sila koje postoje između negativno naelektrisanih gljivica i polimerne površine [23].

Veruje se da je jedan od etioloških faktora za nastanak proteznog stomatitisa trauma, koja može nastati usled loše retencije i stabilizacije proteze u ustima. U istraživanju koje su sproveli Zissis i sar. pokazano je da su trauma, izazvana lošom retencijom proteze i dugotrajno nošenje proteze bili značajni faktori koji su uticali na razvoj zapaljenja [24]. U studiji Emami i sar. testirana je i prihvaćena hipoteza da povećan okluzalni ptitisak može izazvati traumu sluzokože, što rezultuje zapaljenskom reakcijom koja dalje može stvoriti takvu sredinu koja favorizuje razvoj mikroorganizama odgovornih za nastanak proteznog stomatitisa [25].

U rizične faktore za razvoj proteznog stomatitisa ubraja se i loša oralna higijena. Brojne studije potvrdile su jasnu vezu između loše oralne higijene i povećanog rizika za nastanak i prevalenciju zapaljenskog procesa[26-31].

trapped by irregular surface of the prosthesis, even after its cleaning. Polishing of prosthesis makes their surfaces smooth and reduce the initial attachment and accumulation of microorganisms [18]. This has been shown in the paper of Sesma et al [19]. In addition, an important factor is the age of prosthesis, because it is more difficult to maintain hygiene if prosthesis are worn for a long time and there is a tendency towards to porosity of the denture base, which favors the occurrence of infection [20].

The role of the saliva in the colonization of *C. albicans* to the surface of the prosthesis is still controversial. Some studies suggest that it reduces the adhesion of *C. albicans*. On the other hand, saliva possesses defensive molecules as lysozyme, lactoferrine, calpro-tectin, IgA that decrease the adhesion of *Candida* to the oral surfaces [3]. The decrease or the complete absence of saliva in individuals with xerostomia induces the change and the imbalance of the normal microbial communities favoring the proliferation of bacteria as *Staphylococcus* aureus that inhibits the normal adaptation of the commensals [21].

In the space between the gingival surface of the base of the upper denture and palatal mucosa due to good retention, salivary flow is reduced, as well as the dynamics of change in pH of saliva, that leads to the formation of acidic environment and favorable conditions for the occurrence of infections. In the lower jaw dentures, valve effect is weaker so that it changes the pH and flow of saliva, and the development of denture stomatitis in the lower jaw is an uncommon phenomenon.

On the other hand, the hydrophobicity of the prosthesis surface affects the adherence of *C. albicans*, in the way that hydrophobic surfaces decrease the adherence of the cell [22]. As for the electrostatic interaction, yeasts, which surface has a positive charge, are more adherent unlike the repulsive forces which exist between the negatively charged yeast and polymer surfaces [23].

It is believed that one of the etiological factors for the development of denture stomatitis is trauma, which may occur due to poor retention and stabilization of the prosthesis. In a study conducted by Zissis et al., it has been shown that the trauma caused by poor denture retention and long-term wear of dentures were significant factors that lead to the development of inflammation [24].

Ove studije su utvrdile da među osobama koji nose zubne proteze većinu čine starije osobe koje oralnu higijenu održavaju samo pranjem proteze. Međutim, ovo nije dovoljno za održavanje pravilne higijene proteze kao i za prevenciju akumulacije oralnog biofilma. Poželjno je dodatno koristiti komercijalno dostupne dezinfekcione rastvore ili potapati protezu u razblažen rastvor natrijum hipohlorita. Neadekvatno čišćenje proteza dovodi do brzog razvoja i akumulacije biofilma. Biofilm sadrži bakterije i gljivice koje ostaju na površini proteze i tako mogu kolonizovati oralnu sluzokožu, a sam biofilm i gljivice imaju već pomenutu ulogu u razvoju zapaljenja sluzokože u proteznom stomatitisu [32,33]. Takođe, nošenje proteze noću u toku spavanja može biti povezano sa lošom oralnom higijenom i sa razvojem proteznog stomatitisa [26-31].

Materijal od kojeg se izrađuje zubna proteza može otpuštati određene hemijske sastojke koji izazivaju alergijsku reakciju u vidu lokalizovanog ili generalizovanog stomatitisa. Ova reakcija se povezuje sa prisustvom rezidualnog monomera iz akrilatnih smola, hidrokinon peroksida, dimetil-p-toluidina ili metakrilata u protezi. Reakcija preosetljivosti se češće javlja ukoliko se za izradu proteze koristi hladni, tj. autopolomerizujući umesto toplopolimerizujući akrilat [34].

Pored lokalnih etioloških faktora, razvoj proteznog stomatitisa može biti potpomognut nekim opštim faktorima. U ove faktore spadaju dijabetes melitus, pušenje, dugotrajna primena antibiotika i kortikosteroida, radio i hemoterapija, neadekvatna ishrana – nedostatak vitamina B12, folata i gvožđa, psihotropni lekovi [35-37]. Na postojanje sistemskog oboljenja, kao što je dijabetes melitus, može ukazati prisustvo i oralne kandidoze [38]. Kandidoza kod dijabetičara koji nose zubnu protezu povezuje se sa lošim održavanjem oralne higijene i dugotrajnim nošenjem proteze. Takođe, smatra se da je rast gljivica olakšan zbog povećanih vrednosti šećera u tkivnim tečnostima. Povećana glikosijalija utiče na pojačanu adheziju gljivica za epitelne ćelije, pa se prisustvo gljivica povezuje sa kvantitativnim (kserostomija) i kvalitativnim poremećajem pljuvačne sekrecije i oslabljenim ćelijskim imunitetom [39]. Još jedan faktor koji favorizuje gljivičnu infekciju kod dijabetesnih bolesnika je poremećena funkcija neutrofilnih leukocita [37].

In their study, Emami et al. tested and accepted the hypothesis that increased occlusal pressure can cause trauma of mucosa, resulting in inflammatory reaction which can create an environment that favors the growth of microorganisms responsible for the formation of denture stomatitis [25].

The risk factor for the development of denture stomatitis is poor oral hygiene. Numerous studies have confirmed a clear relationship between poor oral hygiene and increased risk for the occurrence and prevalence of inflammatory process [26-31]. These studies have found that the majority of people who wear dentures are elderly who maintain the oral hygiene by washing dentures. However, this is not enough to maintain the proper hygiene of dentures nor to prevent the accumulation of the oral biofilm. It is preferable to use additional commercially available disinfecting solutions or immerse the denture in diluted sodium hypochlorite solution. Inadequately cleaning of dentures accelerates the development and accumulation of pathogenic denture biofilm. Biofilm contains bacteria and yeasts that reside on the denture surfaces and can also colonize the oral mucosa. The role of biofilm and yeast biofilm in the development of inflammation have already been mentioned [32,33]. Also, wearing dentures overnight may be associated with poor oral hygiene and the development of denture stomatitis [26-31].

The material of which denture is made can release certain chemical substances that cause an allergic reaction in the form of localized or generalized stomatitis. This reaction may be related to the presence of residual monomers from the acrylic resins, the presence of hydroquinone peroxide, dimethyl-p-toluidine or methacrylate in the prosthesis. Hypersensitivity reactions more commonly occur with cold or autopolymerized resins than with heat-cured denture-base materials [34].

In addition to local etiological factors, the development of denture stomatitis can be aided by some general factors. These factors include diabetes mellitus, smoking, long-term use of antibiotics and corticosteroids, radio - and chemotherapy, inadequate nutrition - lack of vitamin B12, folate and iron, psychotropic drugs [35-37]. The presence of oral candidiasis may be a sign of systemic diseases, such as diabetes mellitus [38]. Candidiasis in diabetic patients who wear dentures

Terapija proteznog stomatitisa

S obzirom da na razvoj proteznog stomatitisa utiče veliki broj faktora, terapija ovog zapaljenja je kompleksna. Osnova uspeha svakog terapijskog postupka je dobra oralna higijena i higijena zubnih proteza. Ujedno, dobra oralna higijena se ubraja i u osnovne preventivne mere koje smanjuju mogućnost nastanka zapaljenskog procesa.

Jako je važno isključiti sve lokalne faktore koji favorizuju rast gljivica, kao što su pušenje i nošenje proteza tokom noći. Zubne proteze treba skidati tokom spavanja, a sa druge strane, dok su u ustima, bitno je da su dobro retinirane i stabilne kako ne bi izazvale traumu na oralnoj sluzokoži [3]. Ukoliko proteze nisu dobro retinirane, u terapijske svrhe može se vršiti podlaganje proteze tkivnim kondicionerima sa antifugalnim agensima ili, ako je potrebno, mogu se izraditi nove proteze [40,41]. Tkivni kondicioneri sa antifugalnim agensima, kao što je nistatin, ostvaruju inhibitorni efekat na rast *C.albicans, C.krusei i C.tropicalis* [41]. U nekim studijama je potvrđeno da se prevalencija proteznog stomatitisa može redukovati ukoliko se u donjoj vilici proteze stabilizuju primenom implanata, jer se na ovaj način sprečava trauma oralne sluzokože kod bezubih pacijenata [25].

Što se tiče higijene proteza, ona podrazumeva njihovo pranje uz upotrebu četkice i paste, kao i potapanje proteze tokom noći u rastvor antiseptika. Među antisepticima najčešće se koriste razblaženi rastvor natrijum hipohlorita (0,1%), u koji se proteza potopi u trajanju od 15-30 minuta, kao i rastvor hlorheksidin glukonata [42]. Rastvor hlorheksidina u koncentraciji od 0,2% može se koristiti za ispiranje usne duplje tri puta dnevno, što značajno smanjuje akumulirani biofilm, ali ne utiče značajno na redukovanje količine *C.albicans* [34]. Jedan od načina dezinfekcije proteze, koji se u nekim studijama pokazao efikasnim, jeste izlaganje proteze mikrotalasnim zracima [43].

Kada se kao uzročnik proteznog stomatitisa izoluju gljivice iz roda *Candida*, potrebno je sprovesti terapiju antimikoticima. Ova terapija podrazumeva lokalnu ili sistemsku primenu antimikotičnih lekova. Njihov mehanizam delovanja podrazumeva inhibiciju enzima i puteva bitnih za sintezu ćelijske membrane, menjanje propustljivosti ćelijske membrane i metabolizma RNK i DNK, ili unutarćelijsku akumulaciju peroksida koji toksično deluje na gljivice.

is associated with poor oral hygiene and long wearing of dentures. Also, it is considered that the growth of yeast is promoted by elevated tissue fluid glucose levels. Moreover, besides the presence of a high concentration of salivary glucose, low salivary secretion may enhance the growth of yeasts and their adherence in epithelial oral cells [39]. Another factor that favors yeast infection in diabetic patients is impaired function of neutrophils, particularly in the presence of glucose[37].

The therapy of denture stomatitis

Due to the etiology of the development of denture stomatitis, which is multifactorial, the therapy of inflammation is complex. The basis of success of any therapeutic procedure is good oral hygiene and hygiene of dentures. At the same time, this is the one of the basic and preventive measures that decreases the possibility of inflammatory processes.

It is important to exclude all local factors that favor the growth of yeast, such as smoking and wearing dentures overnight. Dentures, during sleep, should be removed; on the other hand, it is essential that they are well retained and stable to avoid trauma of the oral mucosa[3]. If prosthesis are not well-retained, therapy can be performed by relining dentures with conditioners with antifungal agents, or, if it is necessary, a new prostheses can be created [40,41]. Tissue conditioners with antifungal agents, such as Nystatin, have an inhibitory effect on the growth of *C. albicans, C.tropicalis and C.krusei* [41]. In some studies, it was confirmed that the prevalence of denture stomatitis can be reduced if the mandibular denture is stabilized by using implants, because in this way it prevents trauma of oral mucosa in edentulous patients [25].

Hygiene of dentures includes their washing, using brushes and paste, as well as the immersing of dentures overnight in an antiseptic solution. Among the most commonly used antiseptics are diluted sodium hypochlorite solution (0.1%), in which the prosthesis is submerged for a period of 15-30 minutes, and a solution of chlorhexidine gluconate [42]. A solution of chlorhexidine at a concentration of 0.2% can be used for rinsing the oral cavity three times a day, which significantly reduces the accumulated biofilm, but it does not significantly affect the reduction of the number of *C. Albicans* [34]. The exposure of prosthesis to microwave radiation is another way for the disinfection of dentures, which has proved effective in some studies [43].

Efekat antimikotičnih lekova zavisi od koncentracije leka i osetljivosti različitih sojeva [8]. Tretman obično započinje lokalnom primenom ovih preparata, dostupnih u obliku gela, pastila, krema i oralnih suspenzija [3]. Najviše primenjivane su suspenzije na bazi nistatina, amfotericina B, flukonazola i mikonazola. Sa druge strane, klotrimazol se primenjuje u obliku kreme ili rastvora, s tim da krema ima i antistafilokoknu aktivnost. Uglavnom, svi lekovi dovode do prestanka simptoma nakon primene od 12 do 14 dana. Klotrimazol (1% krem) se primenjuje samo lokalno, jer sistemski aplikovan pokazuje toksični efekat na nervni sistem i gastro-intestinalni trakt. Ekonazol postoji samo u obliku za lokalnu primenu, a mikonazol se u obliku 2-4% kreme primenjuje lokalno [44-46]. Sistemski antimikotični lekovi se primenjuju kod pacijenata sa posebnim potrebama i kod imunokompromitovanih pacijenata, kao i kod pacijenata kod kojih lokalna terapija nije dala rezultate [3]. Flukonazol je slabo toksičan i široko se primenjuje u obliku kapsula (50-100 mg); itrakonazol se primenjuje u obliku kapsula u dozi od 100 mg, a ketokonazol u dozi od 200-400 mg, jedanput dnevno u toku 14 dana [47]. Ketokonazol je hepatotoksičan i može izazvati srčane aritmije ukoliko se primenjuje u kombinaciji sa makrolidnim antibioticima ili sa antihistaminicima. Amfotericin B se ranije intravenozno primenjivao u tretmanu kandidom izazvanog proteznog stomatitisa, ali je danas njegova primena redukovana zbog nefrotoksičnosti [48]. Amfotericin B za lokalnu primenu u obliku 3% losiona primenjuje se dva puta dnevno, ali ima neprijatan ukus, pa može izazvati gastrointestinalne smetnje poput mučnine, povraćanja i dijareje [34].

Sve više se u terapiji proteznog stomatitisa izazvanog *C.albicans* primenjuju alternativna sredstva koja zamenjuju hemijske preparate i imaju manje neželjenih efekata. To su prirodni preparati na bazi biljaka koji ispoljavaju antibakterijsku i antifugalnu aktivnost, a takođe imaju i antiinflamatorni i antioksidativni efekat [49]. Najčešće se primenjuju etarska ulja i ekstrati biljaka kao što je timijan, čajno drvo, bergamot, limun i grejpfrut. Hammer i sar. su testirali 20 etarskih ulja i pokazali da ulje timijana ima najnižu inhibitornu koncentraciju za *C.albicans* i *E.coli* [50]. U studiji u kojoj je ispitivan efekat etarskih ulja na gljive roda *Candida*, utvrđeno je da ulje timijana ima najveći inhibitorni efekat.

When genus of *Candida* is isolated as a cause of denture stomatitis, it is necessary to prescribe the antifungal therapy. This therapy includes the local or systemic administration of antifungal drugs. Their mechanism of action include the inhibition of various enzymes and pathways that are essential for the synthesis of cell membranes, alteration of the yeast cell membrane permeability, RNA and DNA metabolism change and intracellular accumulation of peroxide which has a toxic effect on yeasts. The effect of antifungal drugs depends of their concentration and the sensitivity of the various strains [8]. Treatment usually begins with local administration of these drugs, that are available in the form of gels, pastilas, creams and oral suspensions [3]. Suspensions are mostly based on nystatin, amphotericin B, fluconazole and miconazole.

On the other hand, clotrimazole is applied in the form of a cream or a solution, and the cream has an antistaphylococcal activity. Basically, all drugs lead to cessation of symptoms after the application of 12 to 14 days. Clotrimazole (1% cream) is applied only locally because it shows a toxic effect on the nervous system and the gastrointestinal tract. Econazole exists only in the form for atopical use; Miconazole is in the form of 2-4% cream and it is applied topically [44-46]. Systemic antifungal drugs are administered to patients with special needs and in immunocompromised patients, and in patients in whom topical treatment has not yielded results. Fluconazole [3], which is widely applied in the form of capsules (50-100 mg), is sparingly toxic and cost-effective; Itraconazole is also applied in the form of capsules at a dose of 100 mg, and ketoconazole is used in a dosage of 200-400 mg, once a day for 14 days [47]. Ketoconazole is hepatotoxic, and can cause cardiac arrhythmia when it is administered in combination with antihistamines or a macrolide antibiotic. Amphotericin B was previously intravenously used in the treatment of Candida-induced denture stomatitis. However, its administration has been reduced because of its nephrotoxic effect [48]. Amphotericin B for topical application in the form of a 3% lotion is applied twice a day, but it has an unpleasant taste and may cause gastrointestinal disturbances such as nausea, vomiting and diarrhea [34].

In the therapy of denture stomatitis caused by *C. albicans*, alternative agents

Pokazano je da terpinen-4-ol (glavna komponenta ulja čajnog drveta) ima fungistatsko i fungicidno dejstvo na izolovane sojeve *C.albicans*, posebno na one sojeve koji su rezistentni na flukonazol [51].

Kao poslednji vid terapije proteznog stomatitisa, a koji je obično namenjen težim slučajevima proteznog stomatitisa, kao što je papilarna hiperplazija nepca, može se sprovesti kriohirurški tretman, elektrohirurgija, tretman laserom ili ekscizija promena [20].

Zaključak

Imajući u vidu da je protezni stomatitis najčešća zapaljenska reakcija koja se javlja kod nosioca zubnih proteza i da je u većini slučajeva asimptomatski, poželjno je da se kod ovih pacijenata zakazuju redovne stomatološke kontrole u cilju rane dijagnostike i lečenja promena. Izbor terapije zavisi od uzroka promena. Kod većine pacijenata uklanjanje mehaničkih i traumatskih faktora, kao i dobra oralna i higijena zubnih proteza, daje zadovoljavajuće rezultate.

Zahvalnost

Autori nemaju finansijsku korist ili sukob interesa.

which replace chemical preparations and have less side effects are increasingly applied. These are natural products based on herbs that exhibit antibacterial and antifungal activity, but also have antiinflammatory and antioxidant effects [49]. Most commonly applied are essential oils and extracts of plants such as thyme, tea tree, bergamot, lemon and grapefruit. Hammer et al. tested twenty essential oils and showed that thyme oil has the lowest inhibitory concentration for *C. albicans* and E. coli [50]. In a study that examined the effects of essential oils on the yeasts of the genus *Candida*, it was found that thyme oil has the highest inhibitory effect. It has been shown that the terpinene-4-ol (major component of tea tree oil) has fungistatic and fungicidal action on the isolated C. albicans strains, particularly those strains which are resistant to fluconazole [51].

The last form of therapy of denture stomatitis, which is usually designed for severe cases of denture stomatitis, such as papillary hyperplasia of the palate, may include criosurgical treatment, electrosurgery, laser treatment or excision of lesions [20].

Conclusion

Having in mind that the denture stomatitis is the most common inflammatory reaction that occurs in the carriers of dentures, being mostly asymptomatic, it is desirable that these patients schedule regular dental checks for early diagnostics of changes and their treatment. The choice of treatment depends on the cause of a change. In most patients, the removal of mechanical and traumatic factors, as well as good oral hygiene and hygiene of dentures, yields satisfactory results.

Acknowledgements

The authors have no financial benefit or conflict of interests.

LITERATURA /REFERENCE

1. Gendreau L, Loewy ZG. Epidemiology and etiology of denture stomatitis. J Prosthodont 2011;20(4): 251-260.
2. Reichart PA. Oral mucosal lesions in a representative cross-sectional study of ageing Germans. Community Dent Oral Epidemiol 2000; 28: 390–398.
3. Sharma D, Sharma N. Denture stomatitis – a review. IJOCR 2015; 3(7): 81-85.
4. Figueiral MN, Azul A, Pinto E, Foncesa PA, Branco FM. Denture related stomatitis: identification of aetiological and predisponsing factors –A large cohorte. J Or Rehabil 2007; 34: 448-455.
5. Sahebjamee M, Shabestari B, Asadi G, Neishabouri K. Predisposing factors associated with denture induced stomatitis in complete denture wearers. Shiraz Univ Dent J 2011; 11(Suppl): 35-39.
6. Kossioni AE. The prevalence of denture stomatitis and its predisposing conditions in an older Greek population. Gerodontology 2011;28(2):85-90.
7. Mubarak S, Hmud A, Chandrasekharan S, Aiman A. Prevalence of denture-related oral lesions among patients attending College of Dentistry, University of Dammam: A clinico-pathological study. J Int Soc Prev Community Dent 2015;5(6):506–512.
8. Zwiri AMA. The prevalence and associated factors of denture wearing associated oral lesions among dental patients attending college of dentistry clinics n Aljouf University. Eur Sci J 2016; 12(9): 326-332.
9. Ramage G, Tomsett K, Wickers BL, et al: Denture stomatitis: a role for Candida biofilms. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004; 98: 53-59.
10. Mago J, Mittal K, Kaur M, Kaur A, Kaunda M. Denture stomatitis – a case report and an update. World J Pharm Med Res 2016; 2(4): 224-226.
11. Newton AV. Denture sore mouth. Br Dent J 1962; 112: 357-360.
12. Webb BC, Thomas CJ, Willcox MDP, et al: Candida-associated denture stomatitis. Aetiology and management. A review. Part 2. Oral diseases caused by Candida species. Aust Dent J 1998; 43: 160-166.
13. Gasparoto TH, Dionisio TJ, de Oliveira CE, Porto VC, Gelani V, Santos CF, Campaneli AP, Lara VS. Isolation of Candida dubliensis from denture wearers. J Med Microbiol 2009; 58: 959–962.
14. Ramage G, Lopez-Ribot JL. Teehniques for antifungal susceptibility testing of Candida albicans biofilms. Methods Mol Med 2005; 118: 71-79.
15. Figueiral MH, Azul A, Pinto E, Fonseca PA, Branco FM, Scully C. Denture-related stomatitis Identification of aetiological and predisposing factors - a large cohort. J Oral Rehabil 2007; 34: 448–455.
16. Coco BJ, Bagg J, Cross LJ et al. Mixed Candida albicans and Candida glabrata populations associated with the pathogenesis of denture stomatitis. Oral Microbiol Immunol 2008; 23: 377–383.
17. Radford DR, Challacombe SJ, Walter JD. Denture plaque and adherence of Candida albicans to denture-base materials in vivo and in vitro. Crit Rev Oral Biol Med 1999; 10(1): 99-116.
18. Zamperini CA, Machado AL, Vergani CE, Pavarina AC, Giampaolo ET, da Cruz NC. Adherence in vitro of Candida albicans to plasma treated acrylic resin. Effect of plasma parameters, surfaceroughness and salivary pellicle. Arch Oral Biol 2010; 55(10): 763-770.
19. Sesma N, Cruz-Laganá D, Morimoto S, Gil C. Effect of denture surface glazing on denture plaque formation. Braz Dent J 2005; 16(2): 129-134.
20. Shamimul H, Kuldeep. Denture Stomatitis: A Literature Review. JOHS 2015; 6(2): 65-69.
21. Baena-Monroy T, Moreno-Maldonado V, Franco-Martínez F, Aldape-Barrios B, Quindos G, Sanchez-Vargas LO. Candida albicans, Staphylococcus aureus and Streptococcus mutans colonization in patients wearing dental prosthesis. Med Oral Patol Oral Cir Bucal 2005; 10(1): 27-39.
22. Pereira-Cenci T, Del Bel Cury AA, Crielaard W, Ten Cate JM. Development of Candida-associated denture stomatitis: new insights. J Appl Oral Sci 2008; 16(2): 86-94.
23. Salerno C, Pascale M, Contaldo M, Esposito V, Busciolano M, Milillo L, et al. Candida-associated denture stomatitis. Med Oral Patol Oral Cir Bucal 2011; 16(2): 139-143.
24. Zissis A, Yannikakis S. Comparison of denture stomatitis prevalence in 2 population groups. Int J Prosthodont 2006; 19: 621–625.
25. Emami E, de Grandmont P, Rompre PH, et al. Favoring trauma as an etiological factor in denture stomatitis. J Dent Res 2008; 87: 440-444.
26. Marchini L, Tamashiro E, Nascimento DFF, et al. Self-reported denture hygiene of a sample of edentulous attendees at a University dental clinic and the relationship to the condition of the oral tissues. Gerodontology 2004; 21: 226-228.
27. Dikbas I, Koksai T, Calikkocaoglu S. Investigation of the cleanliness of dentures in a university hospital. Int J Prosthodont 2006; 19: 294-298.
28. Khasawneh S, Al-Wahadni A. Control of denture plaque and mucosal inflammation in denture wearers. J Ir Dent Assoc 2002; 48: 132-138.
29. Evren BA, Uludamar A, Işeri U, Ozkan YK. The association between socioeconomic status, oral

hygiene practice, denture stomatitis and oral status in elderly people living different residential homes. Arch Gerontol Geriatr 2011; 53(3): 252-257.
30. Shulman JD, Rivera-Hidalgo F, Beach MM. Risk factors associated with denture stomatitis in the United States. J Oral Pathol Med 2005; 34(6): 340-346.
31. Kulak-Ozkan Y, Kazazoglu E, Arikan A. Oral hygiene habits, denture cleanliness, presence of yeasts, and stomatitis in elderly people. J Oral Rehabil 2002; 29: 300-304.
32. Campos MS, Marchini L, Bernardes LAS, et al. Biofilm microbial communities of denture stomatitis. Oral Microbiol Immunol 2008; 23: 419-424.
33. Coulthwaite L, Verran J. Potential pathogenic aspects of denture plaque. Br J Biomed Sci 2007; 64: 180-189.
34. Pattanaik S, Vikas BVJ, Pattanaik B, Sahu S, Lodam S. Denture stomatitis: A literature review. JIAOMR 2010; 22(3): 136–140.
35. Soysa NS, Ellepola AN. The impact of cigarette/tobacco smoking on oral candidosis: an overview. Oral Dis 2005; 11: 268–273.
36. Soysa NS, Samaranayake LP, Ellepola AN. Cytotoxic drugs, radiotherapy and oral candidiasis. Oral Oncol 2004; 40: 971–978.
37. Soysa NS, Samaranayake LP, Ellepola AN. Diabetes mellitus as a contributory factor in oral candidosis. Diabet Med 2006; 23: 455–459.
38. Obradović R, Kesić Lj, Petrović M, Stanković I. Differential diagnosis and therapeutic characteristics of oral candidiasis. Acta Stomatologica Naissi 2013; 29(68): 1315-1323.
39. Obradović R, Kesić Lj, Pejčić A, Petrović M, Živković N, Živković D. Diabetes mellitus and oral candidiasis. Acta Stomatologica Naissi 2011; 27(63): 1025-1034.
40. Davenport JC, Basker M, Heath JR, Ralph JP, Glantz PO, Hammond P. Initial prosthetic treatment. Br Dent J 2001; 190: 235-244.
41. Skupien JA, Valentini F, Boscato N, Pereira-Cenci T. Prevention and treatment of Candida colonization on denture liners: a systematic review. J Prosthet Dent 2013; 110(5): 356-362.
42. Farah CS, Lynch N, McCullough MJ. Oral fungal infections: an update for the general practitioner. Aus Dent Jour 2010; 55:(1 suppl): 48–54.
43. Ribiero D, Pavarina A, Dovigo L, Palomari Spolidorio D, Giampaolo E, Vergani C. Denture desinfection by microwave irradiation: A randomized clinical study. J Dent 2009; 37: 666-672.
44. Ellepola ANB, Samaranayake PL. Antimycotic agents in oral candidosis: An Overview: 2. Treatment of Oral Candidosis. Dent Update 2000; 27: 165-174.
45. Sherman RG, Prusinski L, Ravenel MC, Joralmon RA. Oral candidosis. Quintessence International 2002; 33(7): 521-532.
46. Martinez-Beneyto Y, Lopez-Jornet P, Velandrino-Nicolas A, Jornet-Garcia V. Use of antifungal agents for oral candidiasis Results of a national survey. Int J Dent Hyg 2010; 8: 47–52.
47. Dar-Odeh NS, Al-Beyari M, Abu-Hammad OA. The role of antifungal drugs in the management of denture associated stomatitis. Int J Antimicrob Agents 2012; 2(1): 1-5.
48. Vasconcellos AAD, Vasconcellos AAD, Chagas RB, Gonçalve LM. Candida-associated denture stomatitis: Clinical relevant aspects. Clin Microbial 2014;3:4.
49. Petrović M, Kostić M, Kostić M, Krunić N, Igić M, Pešić Z, Otašević S. Therapeutic alternatives of natural compounds in treatment of candida-associated denture stomatitis. Acta Medica Medianae 2014; 53(1): 73-79.
50. Hammer KA, Carson CF, Riley TV. A microbial activity of essential oils and other plant extracts. J Apl Microbiol 1999; 86(6): 985-990.
51. Omran S. M, Esmaeilzadeh S, Rahmani Z. Laboratory study of anticandidal activity of thyme, penuyroyal and lemon essential oils by microdilution method. Jundishapur Journal of Microbiology 2010;3(4):161-167.

UPUTSTVA AUTORIMA

Acta Stomatologica Naissi je naučni časopis Stomatološke klinike, Medicinskog fakulteta Univerziteta u Nišu, koji publikuje radove iz svih oblasti stomatologije i srodnih medicinskih grana.

Ljubazno molimo autore da pažljivo pročitaju sledeće informacije o pripremi rada i podnošenju istog za štampanje. Radovi koji nisu napisani u skladu sa ovim instrukcijama biće vraćeni autorima sa zahtevom da isprave korekcije pre slanja istog na recenziju. Odbijeni radovi se ne vraćaju sem slika.

UREĐIVAČKA POLITIKA

Časopis publikuje uvodnik, originalne naučne ili stručne radove, klinički relevantne studije, preglede, prikaz slučaja, preliminarna saopštenja, tehničke inovacije, pisma uredniku, članke iz moderne literature, izveštaj o knjigama, novosti i izveštaje sa nacionalnih i internacionalnih kongresa, simpozijuma i ostalih aktuelnih sastanaka, koji nisu prethodno publikovani ili predati za publikovanje na nekom drugom mestu. Svi primljeni radovi biće recenzirani od dva anonimna recenzenta, i kada je to potrebno od statističara. Autori će biti obavešteni o prijemu, odbijanju, ili potrebnoj reviziji za najkasnije 6 nedelja od podnošenja rada. Radovi se ne plaćaju.

JEZIK

Svi predati radovi za štampanje moraju biti napisani na srpskom i engleskom jeziku. Apstrakti treba da budu pripremljeni pored srpskog i na preciznom i gramatički ispravnom engleskom jeziku (US engleski stil) (videti niže). Izbegavati korišćenje latinskih izraza; ako su potrebni staviti ih u zagrade.

ETIKA

Kada se radi o eksperimentima na humanom materijalu ili pacijentima, ukazati da li je primenjeni postupak u skladu sa etičkim standardima odgovornog komiteta za ljudske eksperimente ili sa Deklaracijom iz Helsinkija (1964, amandmani iz 1975 i 1983) Svetske medicinske asocijacije.

GENERALNE INSTRUKCIJE

PRIPREMA RADA

Radovi treba da budu napisani na A4 formatu sa duplim proredom, obezbeđujući 25 mm margine. Samo jedna kopija rada treba da sadrži prezime i prvo slovo autorovog imena u gornjem desnom uglu. Broj stranica rada počinje sa naslovnom stranom kao strana 1 i nastavlja se sa ređanjem.

NASLOVNA STRANA

Gornji deo naslovne strane treba da sadrži: a) puni naslov rada (velikim slovima), b) puna imena (prvo ime, srednje slovo ako je primenljivo i poslednje ime) svih autora bez akademskih titula, c) nazivi institucija i d) radni naslov od ne više od 10 reči. Na dnu naslovne strane molimo da ukažete na ime autora odgovornog za korespondenciju, sa akademskim zvanjem, poštanskom adresom, telefonskim i fax brojevima i E-mail adresom.

Sledeća strana počinje samo sa naslovom, i dalje se nastavlja sa tekstom. Tekst treba da bude podeljen u delove sa naslovima: uvod, pacijenti/materijal i metod rada, rezultati, diskusija, zaključci, zahvalnost i literatura. Za tabele, figure (slike) i legende vidi deo Tabele I Figure.

Poželjno je da se koriste reči prikladne za indeksiranje i pretraživanje. Ako takvih reči nema u naslovu, poželjno je da se naslovu doda podnaslov.

Ako je članak u prethodnoj verziji bio izložen na skupu u vidu usmenog saopštenja (pod istim ili sličnim naslovom) podatak o tome treba da bude naveden u posebnoj napomeni pri dnu prve strane članka.

APSTRAKT I KLJUČNE REČI

Originalni radovi moraju da sadrže strukturni apstrakt od 250 reči, podeljenih na sledeća 4 paragrafa:

Uvod: opisuje problem o kome se radi u radu

Materijali i metode: opisuje kako je istraživanje sprovedeno

Rezultati: opisuje primarno rezultate

Zaključak(ci): saopštenje autora o zaključcima proisteklim iz rezultata, i implicira njihovu kliničku primenljivost.

Strukturni apstrakti nisu potrebni kod uvodnika i pisma. Ispod apstrakta stoje ključne reči i to tri do pet. Ključne reči mogu biti uzete samo iz Medical Subjects Headings (MeSH).

Apstrakt treba da bude preveden i na engleski jezik (US style), sa naslovom, imenima autora, institucija i ključnim rečima.

TABELE I FIGURE

Svaka tabela sa jasnim naslovom na srpskom i engleskom treba da bude otkucana sa duplim proredom na odvojenom papiru. Obeležiti brojevima tabele jednu za drugom kako nailaze posle prvog navođenja u tekstu (obeležavaju se arapskim brojevima). Dati svakoj kolumni kratko ili skraćeno zaglavlje. Staviti objašnjenja u legendama svih nestandardnih skraćenica korišćenih u tabeli. Za jedinice i merenja vidi odeljak niže. Ne koristiti unutrašnje horizontalne i vertikalne linije. Staviti sve tabele na kraju vašeg fajla. Uvek odvojiti posebne kolumne upotrebom tabulatora, a ne upotrebom razmaknice, tabele moraju biti u tekst formatu.

Linijski prikazani dijagrami i ilustracije (fotografije, fotomikrografije itd.), trebaju biti osmišljene kao figure. Oni takođe treba da budu smešteni na odvojenom listu papira i numerisani jedan za drugim arapskim brojevima u saglasnosti sa prvim koji je citiran u tekstu. Figure treba da budu profesionalno nacrtane i fotografisane. Svaka figura treba da bude etiketirana pozadi ukazujući broj figure, prezime i prvo slovo imena autora, i vrh figure. Fotografije treba da se daju u dva primerka. Kolor fotografije ce se štampati samo u dogovoru sa urednikom ili ako autor sam snosi troškove. Fotomikrografije moraju imati obeleženu unutrašnju razmeru, i simbole, i strelice ili slova treba da su u kontrastu sa pozadinom. Na fotografijama pacijenata mora se sakriti identitet, osim ako se pacijenti u pismenoj formi slože sa objavljivanjem njihovih fotografija sa identitetom. Ukoliko ste pozajmili ili već publikovali negde fotografije priložite i pismenu dozvolu za reprodukovanje. Naslovi i detaljna objašnjenja fotografija treba da budu data u legendama. Ako su korišćeni simboli, strelice, brojevi ili slova za identifikaciju delova slike objasniti svaku jasno u legendi.

ZAHVALNOSTI

Priznanja i zahvalnosti prethode literaturi specificirajući generalnu podršku kao i odeljenje i ime šefa odeljenja, priznanja tehničkoj pomoći i konačno finansijskoj i materijalnoj pomoći. Navesti naziv i broj projekta, odnosno naziv programa u okviru koga je nastao članak i naziv institucije koja je finansirala projekat, u posebnoj napomeni pri dnu prve strane članka

LITERATURA

Autori su odgovorni za tačnost literaturnih podataka. Reference treba da budu na posebnom listu i delu odmah iza teksta. Samo reference bitne za studiju mogu biti citirane. Kada je citiranje literature neophodno primeniti Vankuver stil. Na posebnom listu se navode citati referenci koji su označeni rednim brojevima po redosledu u kome se pojavljuju u tekstu i svaki citat odgovara brojevima koji sadrži navedenu referencu. Primeri tačnih oblika referenci :

RADOVI U ČASOPISIMA

1. Standardni članak u časopisu (lista svih autora, ali ako je broj veći od šest citirati tri i dodati et al): Glass DA, Mellonig JT, Towle HJ. Histologic evaluation of bone inductive proteins complexed with coralline hydroxyapatite in an extraskeletal site of the rat. J Periodontol 1989; 60:121–125.

2. Organizacija kao autor: Federation Dentaire Internationale. Technical Report No. 28. Guidelines for antibiotic prophylaxis of infective endocarditis for dental patients with cardiovascular disease. Int Dent J 1987;37:235.

3. Nije dat autor: Coffee drinking and cancer of the pancreas (editorial).BMJ 1981;283:628

4. Volumen sa suplementom: Magni R, Rossoni G, Berti R, BN52021 protect guinea pig from heart anaohylaxis. Pharmacol Res Commun 1988; 20 Suppl 5:75–8.

Knjige ili druge monografije

5. Lični autor (i): Tullman JJ, Redding SW. Systemic Disease in Dental Treatment. St.Louis: The CV Mosby Company;1983:1–5.

6. Poglavlje u knjizi: Rees TD. Dental management of the medically compromised patient. In: McDonald RE, Hurt WC,Gilmore HW, Middleton RA, eds.Current Therapy in Dentistry, vol.7. St. Louis: The CV Mosby Company; 1980:3–7.

7. Disertacije i teze: Teerakapong A. Langerhans Cells in human periodontally healthy and diseased gingiva. (Thesis). Houston, TX: University of Texas; 1987.92 p.

Ostali publikovani materijal

8. Novinski članak: Shaffer RA.Advances in chemistry are starting to unlock mysteries of the brain. The Washington Post 1989 Ang 7; Sect. A:2 (col. 5).

Reference-elektronski citati

9. On line časopisi bez podataka o volumenu i strani. Berlin JA , Antman EM. Advantages and limitations of metaanalytic regressions of clinical trials data. Online J Curr Clin Trials (serial online). June 4;doc 134. Accessed July 20, 2000.

10. Online časopisi sa podacima o volumenu i strani. Fowler EB, Breault LG. Ridge augmentation with a folded acellular dermal matrix allograft: A case Report. J Contemp Dent Pract (serial online). 2001;2(3):31–40. Available from: Procter&Gamble Company, Cincinnat, OH. Accessed December 15, 2001.

11. World Wide Web.Centers for Disease Control and Prevention. Preventing emerging infectious diseases: Addressing the problem of antimicrobial resistance. Available at: http://www.cdc.gov/ncidod/emergplan/antiresist/. Accessed November 5, 2001.

JEDINICE MERE

Sva merenja treba da budu izražena u terminima Internacionalnog Sistema Jedinica (Si).

SKRAĆENICE I SIMBOLI

Ako se koriste nestandardne skraćenice potrebno je prilikom prvog korišćenja celog izraza u tekstu dati njegov puni naziv, a zatim u daljem tekstu koristiti skraćenicu. Nazivi simptoma, znakova i bolesti, kao i anatomski i histološki detalji ne mogu se skraćivati.

OFFPRINTS

Korespondirajući autori svih tipova radova izuzev pisama, novosti i pregleda knjiga primiće 1 broj časopisa oslobođena plaćanja.

SIMBOLI ZA OZNAČAVANJE (FUSNOTE)

Mogu se koristiti samo za identifikaciju zapošljenja autora, za objašnjenje simbola u tabelama i ilustracijama itd. Koristite sledeće fusnote: *,&, #,**, itd.

PREDAVANJE RADOVA

Poslati 3 kopije rada i elektronsku verziju (CD-ROM, E-mail). Kopije rada i sav sadržaj treba spakovati u tvrdu kovertu kako bi se sprečilo ostećenje za vreme poštanskog saobraćaja. Radovi moraju biti potkrepljeni sa zatvorenim pismom potpisanim od svih autora. Ono mora da sadrži: a) izjavu da je rad pročitan i odobren od svih autora; b) informaciju o prethodnoj ili dupliciranoj publikaciji ili davanju rada na drugom mestu ili nekog njenog dela ranije; c) izjavu o finansijskim ili drugim vezama koje mogu dovesti do sukoba interesa; d) ime, adresu i broj telefona autora za korespondenciju koji je odgovoran za komunikaciju i korespondenciju; e) izjavu da su klinička i eksperimentalna istraživanja sprovedena u skladu sa institucijskim etičkim komitetom ili sa Helsinškom deklaracijom. Sem ovoga, pismo treba da sadrži i obaveštenje o vrsti rada i da li autori plaćaju ekstra cenu za kolor reprodukcije.

Radovi se mogu poslati na sledeću adresu:

Acta Stomatologica Naissi

Sekretari: Prof. dr Saša Stanković, Mr. sci dr Miloš Tijanić

Klinika za Stomatologiju

Bul. Zorana Đinđića 52

18000 Niš, Srbija

E-mail: petras@open.telekom.rs tijanicm@yahoo.com

Predavanje materijala direktno uredniku ili bilo kom članu uređivačkog odbora otežaće i odužiće proces recenzije i prijema rada za štampanje.

TEHNIČKE INSTRUKCIJE ZA ELEKTRONSKO SLANJE RADOVA

Skladištenje informacije: CD-ROM u Windows XP ili veći format. Software: radovi na disku treba da budu u Word-u za Windows. Etiketa: Napišite prvo ime autora na nalepnici CD-a, zajedno sa imenom i verzijom korišćenog word procesora. Označiti sve CD sadržajem figura, dijagrama itd, sa imenom prvog autora, imenom fajla, formatom i sabijenim šemama ako su korišćeni. Fajlovi: priložiti tekst i tabele svakog rada kao pojedinačni fajl, ali stavite sve figure, grafikone itd., u odvojenim fajlovima. Dozvoljeni grafički formati su EPS i TIF. Veličina figura treba da bude 8,5 cm ili 18,0 cm u rezoluciji od minimalno 300 dpi. Molimo Vas da pošaljete originalne fotografije, ne šaljite fotokopije. Format: unosite svoj tekst bespreKidno, samo umetnuti hard return na kraju paragrafa ili poglavlja, podnaslova, lista itd. Ne upotrebljavajte softwareski plan stranica. Molimo Vas da koristite Times New Roman 12 font za Word za Windows. Neku reč ili frazu u tekstu koju želite da izdvojite označite kroz rad u italic pismu. Boldirajte ono što se koristi uzastopno u tekstu za određene matematičke simbole, na primer, vektori. Molimo da proverite disk na virus i verifikujte da on sadrži ispravan fajl.

PODNOŠENJE REVIDIRANIH ČLANAKA

Autori mogu predati svoje revidirane radove uključujući tabele i figure na CD-u sa PC ili Mac fajlom. Vratiti revidirane radove sa celokupnim materijalom na istu adresu sekretarijata.

INSTRUCTIONS TO AUTHORS

Acta Stomatologica Naissi is a scientific journal of the University of Niš, Faculty of Medicine and Clinic of Stomatology, which publishes articles relevant to the science and practice of Dentistry in general and related areas.

Please read carefully the following instructions to authors prior to manuscript preparation and submission. Papers which are not prepared according to the propositions and instructions will be returned to authors for corrections before forwarding them to reviewers. In case of unacceptable articles only illustrations will be returned.

EDITORIAL POLICY

Acta Stomatologica Naissi publishes editorials, original scientific or clinical articles, review articles, preliminary reports, case reports, technical innovations, letters to the editor, articles from up-to-date literature, book reviews, reports and presentations from national and international congresses and symposiums which have not been previously submitted for publication elsewhere. All submitted articles will be reviewed by at least 2 reviewers, and when appropriate, by a statistical reviewer. Authors will be notified of acceptance, rejection, or need for revision within 6 weeks of submission. Articles are not paid for.

LANGUAGE

All submitted articles should be written in bilingual (Serbian and English) language. Abstracts should be written in Serbian and precise and grammatically correct English language, preferably US English. Avoid using Latin terms; however if necessary, put them in parentheses.

ETHICS

When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) or with the Helsinki Declaration (1964, amended in 1975 and 1983) of the World Medical Association.

GENERAL INSTRUCTIONS

PREPARATION

Articles should be written on A4white bond paper size (21x 29.5cm) on one side of the paper only, and double-spaced (including illustration legends and references) providing 25 mm ample margins all around. Only one copy of the manuscript should contain the surname and the author's first name initial in the upper right corner. Manuscripts should be organized as follows: Title Page, Abstract and Key words, Introduction, Patients/Materials and Methods, Results, Discussion, Conclusions, Acknowledgments, References, Figure Legends, Tables, Figures. Title page is numbered as page 1, and all other pages should be numbered conse-quently.

TITLE PAGE

The title page should contain: a) the full title of the article (in upper case); b) first name, middle initial, and last name of each author without the academic degree; c) name of department and institutional affiliation for each author; d) running title of no more than 10 characters. At the bottom of the page, please indicate the name, academic degree and address (including E-mail, telephone and fax number) of the author responsible for correspondence.

It is recommendable to use the words appropriate for indexing and searching. If there are not such words in the title, then subtitle should be added.

If the article in the previous version has been orally exposed (under the same or similar title), such information should be separately noted at the bottom of the first page of the article.

Abstract and Key words

All original abstracts should be submitted with a structured abstract, consisting of no more than 250 words, and the following 4 paragraphs:

Background: Describes the problem being addressed.

Material and Methods: Describes how the study was performed.

Results: Describes the primary results.

Conclusion: Reports what authors have concluded from these results, and notes their clinical implications.

Key words: A maximum of 5 key words drawn from MeSH documentation. Abstract should be translated into English (US style), with the title, name(s) of author(s), institutional affiliation and key words.

TABLES AND FIGURES

Each table with a brief title (on Serbian and English) should be typed double-spaced on a separate sheet of paper. Number tables consecutively (with Arabic numbers) in the order of their first citation in the text. Give each column a short or abbreviated heading. Place explanations in legends of all nonstandard abbreviations which are used in table. For units and measurements see paragraph below. Do not use internal horizontal and vertical rules. Place all tables at the end of your file. Always separate the individual columns using tabulators, not using space bar, i.e. tables must be in text format. Line drawings diagrams and halftone illustrations (photographs, photomicrographs, etc.) should be designated as figures. They should be listed on separate sheet and numbered consecutively with Arabic numerals according to the order in which they have been first cited in the text. Figures should be professionally drawn (not simply typewritten) and photographed. Each figure should be labeled on its back indicated the number of the figure, last name and the first letter of the author, and the topside of the figure. Photographs should be supplied in two copies. Color photographs are published only in case if author himself bears expenses. Photomicrographs must have internal scale markers, and symbols, arrows or letters should contrast with the background. Photographs of patients must conceal their identity unless patients approve the publishing of the photograph in written form. If you borrow or use already published photographs please submit a written permission for reproduction. Permission is not required for the documents in the public domain. Figures will not be returned unless requested. Captions and detailed explanations of the figures should be given in the legends. If symbols, arrows, numbers, or letters are used to identify parts of the figure identity and explain each one clearly in the legend.

ACKNOWLEDGEMENTS

Acknowledgements are positioned before the reference list specifying general support by department chairman, acknowledgements of technical as well as financial and material support. Acknowledgement includes the title and number of the project, i.e. the title of the programme within which the article was composed and the title of the institution funding the project; it should be written as a separate notification at the bottom of the first page of the article.

REFERENCES

Authors are responsible for accuracy of literature data. References should be listed in a separate section immediately following the text. Only references important for the study should be cited. It is necessary to apply Vancouver style. Citations are numbered consecutively in the order in which they appear in the text and each citation corresponds to a numbered reference containing publication information about the source cited in the reference list at the end of the publication. Examples of references are given bellow:

Journals:

1. Standard journal reference. (Note: list all authors if six or less; when seven or more, list only first three and add et al): Glass DA, Mellonig JT, Towle HJ. Histologic evaluation of bone inductive proteins complexed with coralline hydroxyapatite in an extraskeletal site of the rat. J Periodontol 1989;60:121–125.

2. Corporate author: Federation Dentaire Internationale. Technical Report No.28. Guidelines for antibiotic prophylaxis of infective endocarditis for dental patients with cardiovascular disease. Int Dent J 1987;37:235.

3. No author given: Coffee drinking and cancer of the pancreas (editorial). BMJ 1981;283:628

4. Volume with supplement: Magni R, Rossoni G, Berti R, BN52021 protect guinea pig from heart anaphylaxis. Pharmacol Res Commun 1988; 20 Suppl 5:75–8.

Books or other monographs:

5. Personal author(s): Tullman JJ, Redding SW. Systemic Disease in Dental Treatment. St. Louis: The CV Mosby Company; 1983:1–5.

6. Chapter in a book: Rees TD. Dental management of the medically compromised patient. In: McDonald RE, Hurt WC, Gilmore HW, Middleton RA, eds. Current Therapy in Dentistry, vol. 7. St. Louis: The CV Mosby Company; 1980:3–7.

7. Dissertations and thesis: Teerakapong A. Langerhans Cells in human periodontally healthy and diseased gingiva. (Thesis). Houston, TX: University of Texas; 1987.92 p.

Other published material:

8. Newspaper article: Shaffer RA.Advances in chemistry are starting to unlock mysteries of the brain. The Washington Post 1989 Ang 7; Sect.A:2 (col. 5).

References – electronic quotations:

9. Online journals without volume and page information. Berlin JA , Antman EM. Advantages and limitations of metaanalytic regressions of clinical trials data. Online J Curr Clin Trials (serial online). June 4; doc 134. Accessed July 20, 2000.

10. Online journals with volume and page information. Fowler EB, Breault LG. Ridge augmentation with a folded acellular dermal matrix allograft: A case Report. J Contemp Dent Pract (serial online). 2001;2(3):31–40. Available from: Procter&Gamble Company, Cincinnat, OH. Accessed December 15, 2001.

11. World Wide Web.Centers for Disease Control and Prevention. Preventing emerging infectious diseases: Addressing the problem of antimicrobial resistance. Available at: http://www.cdc.gov/ncidod/emergplan/antiresist/. Accessed November 5, 2001.

UNITS OF MEASUREMENTS

All measurements should be reported in terms of the International System of Units (SI)

ABBREVIATIONS AND SYMBOLS

Avoid abbreviations in the text but whenever possible use standard abbreviations. However, if nonstandard abbreviations are used, the full term of which and abbreviation stands for should precede its first use in text. Names of symptoms, signs and diseases, as well as anatomic and histologic characteristics cannot be abbreviated.

OFFPRINTS

The corresponding authors of all types of articles except letters, news and book reviews will receive 1 offprint free of charge.

FOOTNOTES

Footnotes should be used only to identify author affiliation; to explain symbols in tables and illustrations. Use the following symbols: #,†,*,#,$ etc.

SUBMISSION OF MANUSCRIPTS

Send 3 hard copies of the article and its electronic version (diskette, CD-ROM, e-mail). Copies of the articles and all enclosures should be enclosed in hard envelopes to prevent damage during mail handling. Articles must be accompanied by a covering letter signed by all authors. This must include: a) a statement that the article has been read and approved by all authors b) information on prior or duplicate publication or submission elsewhere any part of the work as defined earlier c) statement of financial or other relationships which might lead to a conflict interest d) the name, address and telephone number of the corresponding author who is responsible for communication and correspondence, e) statement that clinical or experimental researches have been performed in accordance with the institutional ethic committee or with Helsinki declaration. So, the letter should contain information about the kind of article, and whether authors pay extra cost for color reproductions.

Submission address:
Acta Stomatologica Naissi
Secretaries: Ass. Prof. dr Saša Stanković, Mr. Sci Dr Miloš Tijanić
Clinic of Stomatology
Bul. Zorana Đinđića 52
18000 Niš, Serbia
E-mail: petras@open.telekom.rs, tijanicm@yahoo.com

Submitting materials directly to any other editor or member of editorial board will delay the review process.

TECHNICAL INSTRUCTIONS FOR ELECTRONIC FILES

Storage medium: CD-ROM in Windows XP or higher format. Software: Articles on disk should be in Word for Windows. Labels: Write the first authors name on the disk label, along with the name and version of the word processor used. Label all CD containing figures etc., with the first authors name , the file name, format and compression schemes (if any) used. Files: Submit the text and tables of each article as a single file, but place all figures, charts etc., in separate files. Allowed graphic formats are EPS and TIF. Size of the figures should be either 8,5 cm or 18,0 cm in resolution of minimum 300 dpi. Please send original photographs, do not send photocopies. Format: Input your text continuously, only insert hard returns at the end of paragraphs or headings, subheadings lists, etc. Do not use page layout software. Please use Times New Roman 12 font for Word for Windows. Any words or phrases in the text that you wish to emphasize should be indicated throughout the paper in italic script. Boldface type that should be used in the running text for certain mathematical symbols, e.g. vectors. Note: Please virus check the disk and verify that it contains the correct file.

SUBMITTING REVISED ARTICLES

Authors should submit their revised articles, including table and figure legends, on a CD using a PC-or Mac-based file. Return the revised article and accompanying materials to the address of secretariat.