Stom Glas S COBISS. SR-ID 8417026 UDC 616.31 ISSN 0039-1743

STOMATOLOŠKI GLASNIK SRBIJE

SERBIAN DENTAL JOURNAL

Vol. 70 • Number 2 • April-June 2023

http://www.stomglas.org.rs

Stom Glas S COBISS. SR-ID 8417026 UDC 616.31

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Vol. 70 • Number 2 • April-June 2023

www.stomglas.org.rs

Adresa uredništva
Srpsko lekarsko društvo
Kraljice Natalije 1
11000 Beograd
Srbija

Telefon: +381 (0)11 409 27 76
Email: stomglas@bvcom.net

Address of the Editorial Office
Serbian Medical Society
Kraljice Natalije 1
11000 Belgrade
Serbia

Phone: +381 11 409 27 76
Email: stomglas@bvcom.net

Časopis izlazi četiri puta godišnje.

The journal is published four times a year.

Finansijsku podršku izdavanju časopisa pružaju Ministarstvo nauke, tehnološkog razvoja i inovacija Republike Srbije i Stomatološka komora Srbije.

The publishing of the Journal is financially supported by The Ministry of Science, Technological Development and Innovation of the Republic of Serbia and Serbian Dental Chamber.

ISSN 0039-1743
ISSN Online 1452-3701
COBISS. SR-ID 8417026
UDC 616.31

www.stomglas.org.rs

Stomatološki glasnik Srbije
Serbian Dental Journal

Izdavač Srpsko lekarsko društvo
Publisher Serbian Medical Society

Osnivač Stomatološka sekcija Srpskog lekarskog društva
Founder Dental Section of the Serbian Medical Society

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ORIGINAL ARTICLE
ORIGINALNI RAD

https://doi.org/10.2298/SGS2302063A
UDC: 616.314.16/.18-08

The impact of irrigation procedures on the bond strength of fiber posts cemented with two different adhesive techniques

Sonja Apostolska, Marija Andonovska, Vasilka Rendžova

University "Ss. Cyril and Methodius", Faculty of Dental Medicine, Department of Restorative Dentistry and Endodontics, Skopje, Republic of Nort Macedonia

SUMMARY

Introduction Endodontically treated teeth are usually weaker due to the loss of tooth structure. As most of the crown of the tooth is destroyed, the most common retention for restoration is application of a fiber-reinforced composite posts in the root canal. In endodontically treated teeth, there are two main problems in the restorative procedure: reduced resistance of the remaining tooth structure and choosing the necessary adequate retention for restoration. The aim of this work was to evaluate the impact of irrigation protocol on the bond strength of two types of fiber posts luted with two different adhesive cements.

Materials and method In this *in vitro* study, 48 single-rooted teeth (incisors, single-rooted second premolars) extracted for orthodontic and periodontal reasons were used. The teeth were divided into the four groups of 12 teeth depending on the type of fiber post used as well as the irrigation agent. Each group was further divided into the two subgroups of six teeth depending on the material and cementation technique.

Results In all cross-sectional statistics the difference appeared only when different types of irrigants were used and they changed bond strength between dentin and bonding material.

Conclusions Irrigation protocol has a significant influence on the bond strength of composite post, independent of the type of post and cementation material used.

Keywords: irrigants; dual-polymerizing cements; composite post

INTRODUCTION

Prognosis of endodontically treated teeth depends not only on the success of endodontic treatment, but also on the type of restoration as these teeth suffer a lot tooth structure loss. Often a post is required as the part of restorative process. Surface treatment of dentin with different irrigants can change chemical and structural composition of dentin, changing its characteristics of permeability and solubility that affect adhesion of materials on the dentin surface [1, 2, 3].

The use of irrigats before starting the bonding procedure can affect adhesion because it changes the properties of the hydrophilic resins. Sodium hypoclorite (NaOCl) and ethylenediamine tetraacetic acid (EDTA) are irrigants commonly used during endodontic treatment. NaOCl is used due to its antibacterial properties, as well as its ability to dissolve the organic part of the smear layer [4, 5, 6]. While EDTA is a chelating agent that binds calcium ions from dentin and enables its easier instrumenting. Adhesion of radicular dentin can be influenced by many factors such as the presence and thickness of smear layer, more difficult light curing, moisture control, the application of adhesive and viscosity of cements [7, 8].

Composite posts reinforced with glass fibers are commonly used for post-endodontic restorations, mainly due to their favorable physical properties, ie. the modulus of elasticity which is similar to dentin. Adhesion of fiber posts to radicular dentin can be influenced by various factors, such as the presence and thickness of residual smear layer after root canal instrumentation, the type of irrigants used, intracanal medications and lubricants, the use of eugenol-based agents, (local analgesics), as well as geometric factors [9, 10]. Materials used for cementing fiber posts and their bonding to the dentin can be influenced by the irrigants used during chemo-mechanical instrumentation of the root canal.

The aim of this work was to evaluate the impact of irrigation protocol on the bond strength of two types of fiber posts luted with two different adhesive cements.

MATERIAL AND METHOD

The tests were carried out at the Clinic for Dental Diseases and Endodontics at the Faculty of Dentistry in Skopje, UKIM and the Faculty of Mechanical Engineering, UKIM, Skopje. For this *in vitro* study, 48 single-rooted teeth (incisors, single-rooted second premolars) extracted for orthodontic and periodontal reasons were used.

During endodontic treatment, root canals were prepared manually using the step-back technique to an apical

Address for correspondence: Sonja APOSTOLSKA, Majke Tereze 43, Skoplje, Makedonija; *sapostolska@yahoo.com*

Table 1. Types of fiber posts, irrigants and adhesive systems used in the study
Tabela 1. Vrste kompozitnih kočića, iriganasa, adhezivnih sistema koji su korišćeni u studiji

	Type of fiber post Vrsta kompozitnih kočića	Irrigants Irigansi	Adhesives Adhezivi
1	FRC Postec Plus Ivoclar Vivadent Inc., Schaan, Liechtenstein	NaOCl	SpeedCEM™ Self-Etch
			Variolink + Excite Total Etch
2	FRC Postec Plus Ivoclar Vivadent Inc., Schaan, Liechtenstein	NaOCl + EDTA	SpeedCEM™ Self-Etch 2.1
			Variolink + Exite Total Etch 2.2
3	Ever Stick GC America	NaOCl	SpeedCEM™ Self-Etch 4.1
			Variolink + Exite Total Etch 4.2
4	Ever Stick GC America	NaOCl + EDTA	SpeedCEM™ Self Etch 5.1
			Variolink + Excite Total Etch 5.2

size of ISO 40. After changing each instrument, root canals were rinsed with 5 ml of 2.5% NaOCl solution. The root canals were dried with paper points (Dentsply Maillefer, Tulsa, Okla., USA) and filled with gutta-percha and AH Plus sealer (Dentsply Caulk, Milford, Del., USA) using cold lateral-compaction technique.

Then the teeth were divided into the four groups of 12 teeth depending on the type of fiber post (FRC Postec Plus /Ivoclar and Ever Stick /GC) as well as the irrigation agent used (NaOCl and NaOCl + EDTA). Each group was divided into the two subgroups of six teeth depending on the material and cementation technique (Table 1).

After obturation, the samples were properly prepared and appropriate composite posts were cemented, depending on the group and subgroup, according to the previously determined work protocol (Table 1). In the subgroup **a**, the posts were cemented with SpeedCEM™(Ivoclar Vivadent Inc., Schaan, Liechtenstein) using the self-etch technique, while in the samples from subgroup **b** the posts were cemented with Variolink (Ivoclar Vivadent Inc., Schaan, Liechtenstein) and Exite as adhesive, using total etch technique.

After endodontic treatment and cementing the composite posts, all samples were firstly invested in plastic molds (FIXI FORM, STRUCTURES), with an internal diameter of 25 mm and a height of 25 mm and are made of PVC (polyvinyl chloride) ISO 3698.

All teeth during casting were secured with bonding wax to the substrate in the center of the mold. Two-component transparent acrylate ORTO POLI (POLIDENT DOO, Slovenija) was used for embedding the samples. We left the embedded samples for 3 hours at room temperature to harden. After removing embedded samples from plastic molds, we proceeded to cutting, i.e., extraction of cross-sections which were further used to examine the bond strength between dentin and post using the Push Out Method. For cutting the sample we used the ISOMET 1000 machine. Examination of the bond strength between the composite post and hard dental tissues, was made with a Push-out test at the Faculty of Mechanical Engineering – Skopje. Obtained results were statistically processed with the statistical package Excel ANOVA 2016 and SPSS.

RESULTS

The results obtained from this study are shown in Table 2. The highest values of the bond strength with the push out test were obtained in the group of teeth where we applied a GC Ever stick posts in combination with Variolink / Excite with Total Etch technique, and NaOCl / EDTA as irrigant (3.12 MPa), while the lowest values were obtained in the

Table 2. The effect of irrigation agents and cementation materials on the bond strength of fiber posts with dentin
Tabela 2. Uticaj sredstava za irigaciju i materijala za cementiranje na čvrstoću veze kompozitnih kočića sa dentinom

	Type of fiber post Vrsta kompozitnih kočića	Irrigants Irigansi	Adhesives Adhezivi	Average value Srednja vrednost μTBS (MPa)	Minimum value Minimalna vrednost (MPa)	Maximum value Maksimalna vrednost (MPa)
1	FRC Postec Plus Ivoclar	NaOCl	SpeedCEM™ Self-Etch	0.76	0.45	1.23
			Variolink + Excite Total Etch	1.118	0.78	1.55
2	FRC Postec Plus Ivoclar	NaOCl + EDTA	SpeedCEM™ Self-Etch	1.36	0.88	1.97
			Variolink +Exite Total Etch	1.59	1.06	2.45
3	GC Ever stick	NaOCl	SpeedCEM™ Self-Etch	1.11	0.55	1.54
			Variolink +Exite Total Etch	1.383	0.86	2.19
4	GC Ever stick	NaOCl + EDTA	SpeedCEM™ Self Etch	1.536	0.96	2.27
			Variolink + Excite Total Etch	2.185	1.09	3.12

Table 3. Assessment of bond strength between the groups according to different irrigation protocol
Tabela 3. Jačina veze između grupa prema različitim protokolima irigacije

Values Vrednosti	N	Mean Srednja vrednost	Std. Deviation Standardna devijacija	Std. Error Standardna greška	95% Confidence Interval for Mean 95% Interval pouzdanosti za srednju vrednost		Minimum Minimum	Maximum Maksimum
					Lower Bound Donja granica	Upper Bound Gornja granica		
NaOCL	24	1.0933	0.43456	0.08870	0.9098	1.2768	0.45	2.19
NaOCL+ EDTA	24	1.6721	0.67013	0.13679	1.3891	1.9551	0.88	3.12
Total Ukupno	48	1.3827	0.63063	0.09102	1.1996	1.5658	0.45	3.12

Table 4. Bond strength between the groups according to the cementing materials and adhesive techniques
Tabela 4. Jačina veze između grupa prema materijalima za cementiranje i adhezivne tehnike

Values Vrednosti	N	Mean Srednja vrednost	Std. Deviation Standardna devijacija	Std. Error Standardna greška	95% Confidence Interval for Mean 95 % Interval pouzdanosti za srednju vrednost		Minimum Minimum	Maximum Maksimum
					Lower Bound Donja granica	Upper Bound Gornja granica		Lower Bound Donja granica
SpeedCEM Self-Etch	36	0.9900	0.51717	0.08619	0.8150	1.1650	0.35	2.27
Variolink Exicte Total-Etch	36	1.2478	0.73574	0.12262	0.9988	1.4967	0.37	3.12
Total Ukupno	72	1.1189	0.64463	0.07597	0.9674	1.2704	0.35	3.12

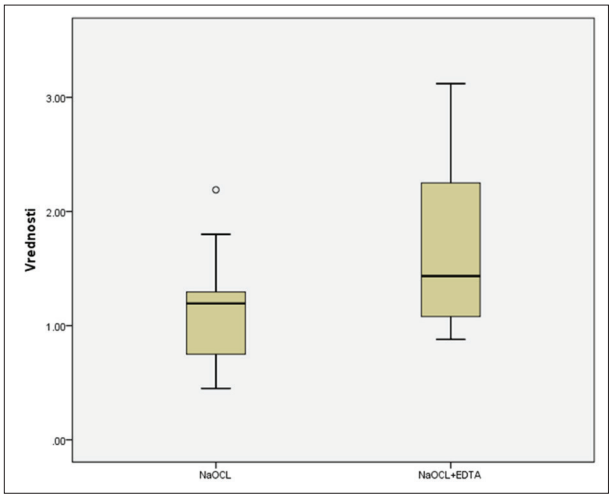

Figure 1. Cross-statistics for bond strength between the groups according to different irrigation protocols
Slika 1. Unakrsna statistika za jačinu veze između grupa prema različitim protokolima irigacije

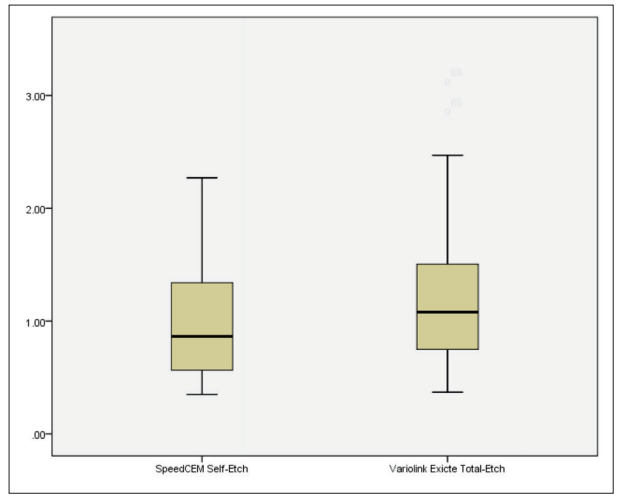

Figure 2. Cross-statistics for bond strength between the groups according to the cementing materials and adhesive techniques
Slika 2. Unakrsna statistika za jačinu veze između grupa prema materijalima za cementiranje i adhezivnim tehnikama

samples where we applied the FRC Postec Plus Ivoclar posts in combination with SpeedCEM™, Self-Etch technique and NaOCl as irrigant (1.23 Mpa).

Table 3 and Figure 1 show the results of cross-statistics between the groups where we used the same fiber posts, the same cements and the same adhesive technique (self-etching and complete etching) but different irrigants (NaOCl with NaOCL and EDTA). We obtained values of p=0.001. As p was < 0.05, that indicates that there is a significant difference between these groups where we used different irrigants.

Table 4 and Figure 2 show the results of cross-statistics of the groups where we used the same composite post FRC Postec Plus Ivoclar and GC Ever Stick, the same irrigants (NaOCl, NaOCl with EDTA), and different cements and adhesive techniques (SpeedCEM with the technique of self-etching, and Variolink cement with total etching technique), where we obtained p=0.090, i.e. p>0.05. This indicates that there was no statistically significant difference in the bond strength between the composite post and dentin, depending on the type of cement used.

DISCUSSION

Several studies have been performed to evaluate the bond strength of fiber posts to root dentin. In intraradicular dentin, a smear layer is attached to the surface after instrumentation with endodontic files [11, 12, 13].

Radicular smear layer acts as a barrier, partially obstructing and sealing dentinal tubules (smear plugs), reducing dentin permeability by up to 86%, making it difficult for substances used as intracanal medication to diffuse, preventing the penetration of the endodontic sealers into the dentinal tubules, as wells hindering the diffusion of monomers into dentinal tubules during adhesive procedure. Thus, its removal is commonly recommended in the literature [14–18].

Irrigation solutions used during endodontic therapy, which have the main objective of cleaning the root canal, can facilitate the reduction and removal of the smear-layer due to their antimicrobial, solvent and chelating actions [12, 19, 20].

Although irrigants used in endodontic therapy are known to affect adhesion, less is known about how they affect the relationship between the tooth and the fiber post.

In our study, a comparison was made between the bond strength of two types of fiber posts cemented with different types of cements, different adhesive techniques, while the root canal was irrigated with different types of irrigants.

The results showed that the use of sodium hypochlorite in combination with EDTA as an irrigant gave the highest bond strength regardless of the cementation technique used and the type of post.

For the groups where sodium hypochlorite and EDTA were used as irrigation agents, we obtained a higher bond strength in the total etching adhesive system than in the self-etching adhesive system, which is still in correlation with the study of Zorba et al., who concluded that the application of 17% EDTA with 5.25% sodium hypochlorite after spatial preparation for composite post increased the strength of self-adhesive cement more than the strength of self-etching cement. The explanation for the reasons was the removal of the secondary residual layer before the cementation of the post and chemical bond of self-adhesive cement [19].

In contrast, Demiryürek et al. concluded that sodium hypochlorite reduces the bond strength of self-etching cement [20]. Similarly, Goracci et al. showed that extracting bond strength of self-adhesive cement can be compared to that of self-etching cement [21].

In all cross-sectional statistics in the groups where we used two types of composite posts and different adhesive techniques, the difference appeared only when different types of irrigants were used and they changed bond strength between dentin and bonding material, thus the first objective where the influence of the effects of irrigation on the bond strength of the two composite posts with the dentin were evident only in the cases of the cross-sectional statistics.

Cementing materials reacted differently only with the application of the different irrigants that we used on the strength of the two types of composite posts.

Solutions such as sodium hypochlorite (NaOCl), ethylenediamine tetra acetic acid (EDTA), chlorhexidine gluconate (CHX) and peracetic acid (PAA) help in removing organic and inorganic elements from smear-layer. They are used during and after endodontic instrumentation, improving the cutting efficiency of the instruments.

According to J.F.C. Lima various irrigation techniques did not exert any influence on the bond strength of intra radicular posts luted with self-adhesive luting agent 24 hours after endodontic treatment when dentin affected by the auxiliary chemical substance was removed before cementation [22].

In cross-sectional statistical analysis in our study, adhesive techniques did not show significant differences in the bond strength of dentin and posts that were used.

The treatment of dentin surface initially implies efficient removal of the root smear layer, followed by the infiltration of adhesive monomers. To this end, etch-rinse (ER)and self-etch (SE) strategies can be used. However, as SE systems are composed of weak acids, they are not as effective as ER systems in removing root smear layer. ER systems are most commonly used adhesive systems for the treatment of root dentin, because in addition to more effective removal of the smear layer, a more uniform demineralization pattern is obtained [23, 24, 25].

CONCLUSIONS

Based on our research we can conclude that irrigation protocol has a significant influence on the bond strength of composite post, independent of the type of post and cementation material used.

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Received: 6.3.2023 • Accepted: 26.5.2023

Uticaj irigacije na jačinu veze kompozitnog kočića pričvršćenog dvema različitim adhezivnim tehnikama

Sonja Apostolska, Marija Andonovska, Vasilka Rendžova

Univerzitet „Sveti Ćirilo i Metodije", Stomatološki fakultet, Klinika za bolesti zuba i endodonciju, Skoplje, Republika Severna Makedonija

KRATAK SADRŽAJ

Uvod Endodontski lečeni zubi su obično oslabljeni zbog gubitka strukture zuba. Često je veći deo krune zuba uništen, pa je najčešća retencija za restauraciju primena kompozitnih kočiča ojačanih vlaknima u kanalu korena. Kod endodontski lečenih zuba postoje dva glavna problema u postupku restauracije, a to su: smanjena otpornost preostale zubne strukture i problem izbora neophodne adekvatne retencije za restauraciju.

Cilj ovog rada je da se proceni uticaj protokola za irigaciju na jačinu veze dve vrste kompozitnih kočića cementiranih sa dva različita adhezivna cementa.

Materijal i metode Za ovu *in vitro* studiju korišćeno je 48 jednokorenih zuba (sekutića, jednokorenih drugih pretkutnjaka) ekstrahovanih iz ortodontskih i parodontalnih razloga. Zatim su zubi podeljeni u četiri grupe od po 12 zuba u zavisnosti od vrste kompozitnog kočića i korišćenog sredstva za irigaciju. Svaka grupa je podeljena u dve podgrupe od po šest zuba u zavisnosti od materijala i tehnike cementiranja.

Rezultati U svim statistikama preseka u grupama gde smo koristili dve vrste kompozitnih kočića i različite adhezivne tehnike, razlika se pojavila samo kada su korišćeni različiti tipovi iriganata koji su menjali jačinu veze između dentina i vezivnog materijala.

Zaključak Na osnovu našeg istraživanja možemo zaključiti da protokol irigacije ima značajan uticaj na jačinu veze kompozitnog kočića, nezavisno od vrste kočića i materijala za cementiranje koji se koristi.

Ključne reči: irigansi; dvojno polimerizirajući cement; kompozitni kočići

UVOD

Prognoza endodontskog tretmana zuba ne zavisi samo od uspeha endodontskog lečenja već i od vrste nadoknade samih zuba. Ti zubi su oslabljeni samim tretmanima i gubitkom strukture zuba. Često je potrebna intervencija sa kočićem u korenu zuba kao deo procesa restauracije.

Površinski tretman dentina različitim irigansama može da promeni hemijski i strukturni sastav dentina, menjajući njegove karakteristike trajnosti i rastvorljivosti koje utiču na adheziju materijala na dentinskoj površini [1, 2, 3].

Upotreba sredstava za irigaciju pre početka postupka vezivanja može uticati na adheziju jer menja svojstva hidrofilnih smola.

Natrijum-hipohlorid (NaOCl) i etilendiamin-tetrasirćetna kiselina (EDTA) supstance su koje se obično koriste tokom endodontskog lečenja. NaOCl se koristi zbog svojih antibakterijskih svojstava, kao i zbog sposobnosti da rastvara organski deo razmaznog sloja [4, 5, 6], dok je EDTA helatni agens koji vezuje jone kalcijuma iz dentina i omogućava njegovu lakšu obradu.

Na adheziju radikularnog dentina mogu uticati mnogi faktori kao što su prisustvo i debljina endodontskog razmaznog sloja, teža svetlosna polimerizacija, kontrola vlage, nanošenje adheziva i viskoznost cementa [7, 8].

Kompozitni kočići ojačani staklenim vlaknima najčešće se koriste za postendodontske nadoknade, uglavnom zbog svojih povoljnih fizičkih svojstava (modul elastičnosti koji je sličan dentinu). Na adheziju vlakana na radikularni dentin mogu uticati različiti faktori, kao što su prisustvo i debljina rezidualnog razmaznog sloja nakon preparacije kanala korena zuba, vrsta korišćenih iriganasa, intrakanalni medikamenti i lubrikanti, upotreba sredstava na bazi eugenola (lokalni analgetici), kao i geometrijski faktori [9, 10].

Na materijale koji se koriste za cementiranje kompozitnih kočića i njihovo vezivanje za dentin mogu uticati irigansi koji se koriste tokom hemomehaničke preparacije kanala korena tokom endodontskog lečenja, a koji su predmet ovog istraživanja.

Cilj ovog rada je da se proceni uticaj protokola za irigaciju na jačinu veze dva tipa kompozitnih kočića cementiranih sa dva različita adhezivna cementa.

MATERIJAL I METODE

Za realizaciju postavljenih ciljeva ispitivanja su obavljena na Klinici za bolesti zuba i endodonciju pri Stomatološkom fakultetu u Skoplju, UKIM i Mašinskom fakultetu UKIM, Skoplje. Za ovu *in vitro* studiju korišćeno je 48 jednokorenih zuba (sekutića, jednokorenih drugih pretkutnjaka) ekstrahovanih iz ortodontskih i parodontalnih razloga.

Tokom endodontskog tretmana, kanali korena su ručno pripremljeni tehnikom *step-back* do apikalne veličine ISO 40. Nakon promene svakog instrumenta, kanali korena su isprani sa 5 ml 2,5% rastvora NaOCl. Kanali korena su osušeni papirnim štiftovima (Dentsply Maillefer, Tulsa, Okla, USA) i punjeni gutaperkom i materijalom za punjenje AH Plus (Dentsply Caulk, Milford, Del., USA) tehnikom hladne lateralne kondenzacije. Zatim su zubi podeljeni u četiri grupe od po 12 zuba u zavisnosti od vrste korišćenog kompozitnog kočića (FRC Postec Plus / Ivoclar i Ever Stick / GC) kao i sredstva za irigaciju (NaOCl i NaOCl + EDTA).

Svaka grupa je podeljena u dve podgrupe od po šest zuba u zavisnosti od materijala i tehnike cementiranja (Tabela 1).

Posle opturacije uzorci su propisno pripremljeni i na njih su cementirani odgovarajući kompozitni kočići, u zavisnosti od grupe i podgrupe, prema prethodno utvrđenom protokolu rada (Table 1). U podgrupi a, kompozitni kočići su cementirani tehnikom samonagrizanja SpeedCEM™ (Ivoclar Vivadent Inc., Schaan, Liechtenstein), dok su u uzorcima iz podgrupe b kompozitni kočići cementirani Variolinkom (Ivoclar Vivadent Inc.,

Schaan, Liechtenstein) i adhezivom Exite, tehnikom totalnog jetkanja.

Nakon endodontskog tretmana i cementiranja kompozitnih kočića, svi uzorci su prvo uloženi u plastične kalupe (Fixi form, konstrukcije) koji su unutrašnjeg prečnika 25 mm i visine 25 mm i izrađeni od PVC (polivinil-hlorida) ISO 3698.

Tokom livenja svi zubi su pričvršćeni voskom za vezivanje za podlogu u centru kalupa. Za ugradnju uzoraka korišćen je dvokomponentni providni akrilat Ortopoli (Polident DOO, Slovenija). Ugrađeni uzorci su ostavljeni tri sata na sobnoj temperaturi da se stvrdnu. Nakon što su ugrađeni uzorci izvađeni iz plastičnih kalupa, prešlo se na sečenje, tj. ekstrakciju poprečnih preseka, koji su dalje korišćeni za ispitivanje jačine veze između dentina i kočića metodom *push out*. Za sečenje uzorka korišćena je mašina ISOMET 1000. Ispitivanje jačine veze između kompozitnog kočića i tvrdih zubnih tkiva urađeno je testom *push out* na Mašinskom fakultetu u Skoplju.

Dobijeni rezultati su statistički obrađeni statističkim paketom Excel ANOVA 2016 i SPSS.

REZULTATI

Rezultati dobijeni iz ove studije prikazani su u Tabeli 2. Najveće vrednosti čvrstoće veze sa testom *push out* dobijene su u grupi zuba gde smo primenili kočiće GC Ever stick u kombinaciji sa Variolink / Excite sa tehnikom totalnog jetkanja i NaOCl / EDTA kao sredstvo za irigaciju (3,12 MPa), dok su najniže vrednosti dobijene u uzorcima gde smo primenili kočiće FRC Postec Plus Ivoclar u kombinaciji sa SpeedCEM™, tehnikom samojetkanja i NaOCl kao irigantom (1,23 Mpa).

Tabela 3 i Grafikon 1 prikazuju rezultate unakrsne statistike između grupa u kojima smo koristili iste kompozitne kočiće, iste cemente i istu tehniku adhezije (samojetkanja i totalnog jetkanja), ali različite iriganse (NaOCl sa NaOCl i EDTA). Dobili smo vrednosti za $p = 0,001$. Kako je $p < 0,05$, to ukazuje da postoji značajna razlika između grupa kod kojih smo koristili različitu irigaciju.

Tabela 4 i Grafikon 2 prikazuju rezultate unakrsne statistike grupa u kojima smo koristili isti kompozitni kočić FRC Postec Plus Ivoclar i GC Ever Stick, iste iriganse (NaOCl, NaOCl sa EDTA) i različite cemente i adhezivne tehnike (SpeedCEM sa tehikom samojetkanja i Variolink cement sa tehnikom totalnog jetkanja), pri čemu smo dobili $p = 0,090$, odnosno $p > 0,05$. Ovo ukazuje na to da ne postoji statistički značajna razlika u jačini veze između kompozitnog kočića i dentina, u zavisnosti od vrste cementa koji se koristi.

DISKUSIJA

Urađeno je nekoliko studija kako bi se procenila jačina veze kompozitnih kočića sa dentinom korena. U intraradikularnom dentinu razmazani sloj se pričvršćuje na površinu nakon instrumentacije endodontskim turpijama [11, 12, 13].

Radikularni razmazni sloj deluje kao barijera, delimično ometa i zaptiva dentinske tubule (razmazne čepove), smanjujući permeabilnost dentina do 86%, što otežava difuziju supstanci koje se koriste kao intrakanalni medikamenti, sprečavajući prodiranje endodontskih materijala u dentinske tubule, kao i ometanje difuzije monomera u dentinske tubule tokom adhezivne procedure. Stoga se njegovo uklanjanje obično preporučuje u literaturi [14–18].

Rastvori za irigaciju koji se koriste u endodontskoj terapiji, a čiji je glavni cilj čišćenje kanala korena, mogu olakšati smanjenje i uklanjanje razmaznog sloja zbog svog antimikrobnog rastvarača i helatnog delovanja [12, 19, 20].

Iako je poznato da irigansi koji se koriste u endodontskoj terapiji utiču na adheziju, manje se zna o tome kako utiču na odnos između zuba i kompozitnog kočića.

U našoj studiji upoređena je jačina veze dve vrste kompozitnih kočića cementiranih različitim vrstama cementa, različitim adhezivnim tehnikama, dok je kanal korena pripremljen različitim vrstama iriganasa.

Dobijeni rezultati su pokazali da je primena natrijum-hipohlorida u kombinaciji sa EDTA kao sredstva za irigaciju dala najveću jačinu veze bez obzira na primenjenu tehniku cementiranja i vrstu kompozitnog kočića.

U grupama u kojima su natrijum-hipohlorit i EDTA korišćeni kao agensi za irigaciju dobili smo veću jačinu veze sa adhezivnom tehnikom sa totalnim jetkanjem nego u adhezivnoj tehnici za samojetkanja, što je još uvek u korelaciji sa studijom Zorbe i sar., koji su zaključili da primena 17% EDTA sa 5,25% natrijum-hipohlorita nakon prostorne pripreme za naknadnu nadogradnju kompozita povećava čvrstoću samolepljivog cementa više od čvrstoće samojetkajućeg cementa. Objašnjenje razloga je uklanjanje sekundarnog zaostalog sloja pre cementiranja kočića i hemijska veza samolepljivog cementa [19].

Nasuprot tome, Demiryürek i sar. zaključili su da natrijum-hipohlorit smanjuje jačinu veze samojetkajućeg cementa [20]. Slično, Goracci i sar. pokazali su da se jačina veze pri ekstrakciji samo cementa može uporediti sa onom samojetkajućeg cementa [21].

U svim statistikama poprečnog preseka u grupama gde smo koristili dve vrste kompozitnih kočića i različite adhezivne tehnike, razlika se pojavila samo kada su korišćeni različiti tipovi iriganasa koji su menjali jačinu veze između dentina i vezivnog materijala, što je bio prvi cilj po kome je uticaj efekata irigacije na jačinu veze dva kompozitna kočića sa dentinom bio evidentan samo u slučajevima statistike poprečnog preseka.

Materijali za cementiranje su različito reagovali samo pri primeni različitih irigansa koje smo koristili na jačini dve vrste kompozitnih kočića.

Rastvori kao što su natrijum-hipohlorit (NaOCl), etilendiamin-tetrasirćetna kiselina (EDTA), hlorheksidin-glukonat (CHX) i persirćetna kiselina (PAA) pomažu u uklanjanju organskih i neorganskih elemenata iz razmaznog sloja. Koriste se tokom i posle endodontske instrumentacije, poboljšavajući efikasnost sečenja instrumenata.

Prema J. F. C. Limi, različite tehnike irigacije nisu imale nikakav uticaj na jačinu veze intraradikularnih kočića fiksiranih samolepljivim sredstvom za cementiranje 24 sata posle endodontskog tretmana kada je dentin pogođen pomoćnom hemijskom supstancom uklonjen pre cementacije [22].

U statističkoj analizi poprečnog preseka u našoj studiji, adhezivne tehnike nisu pokazale značajne razlike u jačini veze dentina i kočića koji su korišćeni.

Tretman površine dentina u početku podrazumeva efikasno uklanjanje razmaznog sloja korena, nakon čega sledi infiltracija

adhezivnih monomera. U tu svrhu mogu se koristiti strategije jetkanja i ispiranja (ER) i samojetkanja (SE). Međutim, kako se SE sistemi sastoje od slabih kiselina, oni nisu tako efikasni kao ER sistemi u uklanjanju razmazanog sloja korena. ER sistemi su najčešće korišćeni adhezivni sistemi za tretman dentina korena, jer se pored efikasnijeg uklanjanja razmaznog sloja dobija i uniformniji obrazac demineralizacije [23, 24, 25].

ZAKLJUČAK

Na osnovu našeg istraživanja možemo zaključiti da protokol irigacije ima značajan uticaj na jačinu veze kompozitnog kočića, nezavisno od vrste kočića i materijala za cementiranje koji se koristi.

ORIGINAL ARTICLE
ORIGINALNI RAD
https://doi.org/10.2298/SGS2302071D
UDC: 616.314.5:616.314.16-73(497.6)

Dentin thickness of the mesio-buccal root wall of the lower first molar using cone beam computerized tomography (CBCT)

Aleksandra Đeri[1], Irena Radman-Kuzmanović[1], Nikola Moravac[2], Milan Tejić[3], Renata Josipović[1], Adriana Arbutina[1], Saša Marin[1], Nataša Trtić[1], Valentina Veselinović[1]

[1]University of Banja Luka, Faculty of Medicine, Department of Dental Medicine, Bosnia and Herzegovina;
[2]ZU "Simić", Banja Luka, Bosnia and Herzegovina;
[3]ZU "Institute of Dentistry", Banja Luka, Bosnia and Herzegovina

SUMMARY

Introduction The aim of this work was to check the average thickness of dentinal wall of the mesio-buccal root of the first lower molar using CBCT axial sections of intact teeth.

Material and method In this cross-sectional study, 100 CBCT images of mandibular first molars from the Promax 3Dent radiology center in Banja Luka, Bosnia and Herzegovina were evaluated. A Planmeca Promax3D Max apparatus (Planmeca, Helsinki, Finland) was used, with a field of view (FOV) of 8 × 8 cm and a resolution of 0.1 mm. Analysis was performed using Romexis Viewer software version 3.1.1 (Planmeca, Helsinki, Finland). At an axial thickness of 0.1 mm, transverse sections with a distance of 1 mm were made below the furcation area (at a magnification ×10) at a distance of 1, 2, 3, 4, 5 mm from the furcation. The data were then analyzed by paired t-test.

Results The thickness of dentinal wall of the mesio-buccal root of the lower first molar was significantly smaller at 2 mm and 3 mm than when measured at 1, 4 and 5 mm ($p < 0.05$). There was no significant difference between measurements at 1, 4 and 5 mm below the furcation area ($p = 0.001$).

Conclusion Due to the small thickness of dentinal wall at 2-3 mm below the furcation in the root canal, caution is required when instrumenting mesio-buccal canal of the lower first molar and correct selection of the instrumentation technique in order to avoid transportation or stripping perforation that may lead to root fracture.

Keywords: cone beam computerized tomography; mandibular molars; dentin thickness

INTRODUCTION

In human dentition, lower first molar is the first of the group of permanent molars that appears in the oral cavity already at the age of six. The early appearance of caries is the reason why it is one of the most frequently treated teeth in restorative and endodontic treatments. In preventative dentistry, lower first molar is the tooth that usually gets fisure sealant applied. From the point of view of dental oclussion, it is a tooth that is responsible for the height of bite, while in orthodontics, it is responsible for maloclussion. Because of the above, knowledge of the morphology of the lower first molar is of great importance in dentistry. Mandibular first molars (MFM) are frequently treated endodontically, with an incidence of 17.0% [1].

The first mandibular molar has a complex morphology, one mesial and one distal root. Mesial roots of mandibular molars usually have two main canals: mesiobuccal (MB) and mesiolingual (ML). The specificity in the anatomical structure of the lower first molars is reflected in the greater concavity of the distal surface of the mesiobuccal root. The thickness of this wall is limited, which should be taken into account during endodontic treatment in order to avoid over-instrumentation and root fracture [2]. Molar furcation area is recognized as one of the most vulnerable areas. The thickness of dentin in distal walls of mesial canals is also mentioned as a risk zone [3, 4, 5].

The appearance of perforations and root fractures during endodontic therapy occurs more often in these areas, and a good understanding of the internal anatomy can help prevent excessive loss of dentin tissue in the danger zone, which can lead to tooth root fractures under functional load [6].

If the clinician neglects the thickness of the dentin wall during the treatment process, he will face perforation of the root canal wall. Perforation occurs especially in curved root canals where canal has been carelessly treated with larger diameter canal instruments [7].

Mesial root of lower first molar is characterized by a wide buccolingual and narrow mesiodistal dimension, while distal root is mostly conical. The morphology of each tooth is individual and may be associated with certain ethnic groups. It was found that the danger zone in Chinese and Indian population is located 4 to 6 mm below the entrance of the root canal and minimum thickness of the distal dentin was located between 1 and 2 mm below furcation [8, 9, 10].

Address for correspondence: Aleksandra ĐERI, Nikole Pašića 36, Banja Luka, Bosna i Hercegovina;
aleksandra.djeri@med.unibl.org

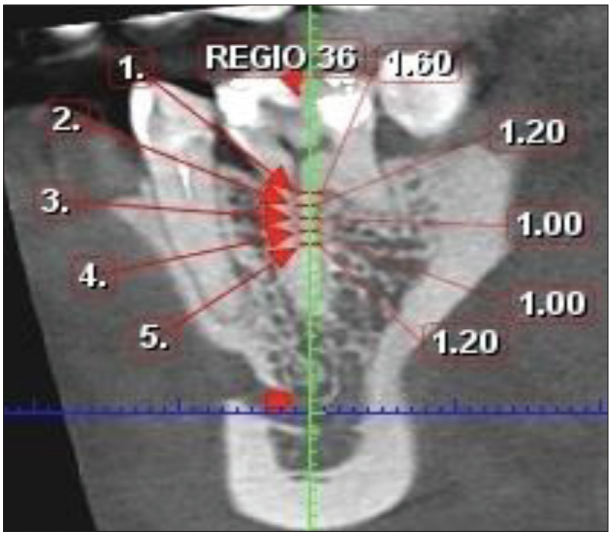

Figure 1. Axial CBCT section with marked dentin thickness measurement sites
Slika 1. Aksijalni CBCT presek sa označenim mestima merenja debljine dentina

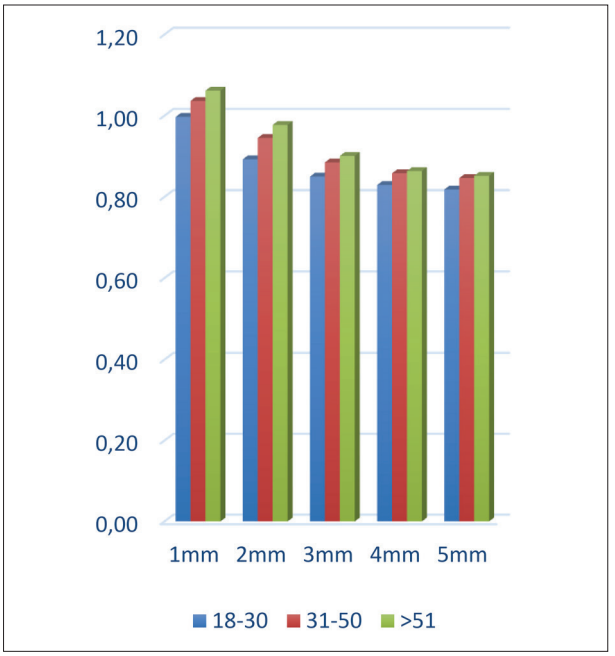

Figure 2. Dentin thickness ratio of MB root in relation to the measurement point in male population
Slika 2. Odnos debljine dentina meziobukalnog korena u odnosu na tačku merenja kod muške populacije

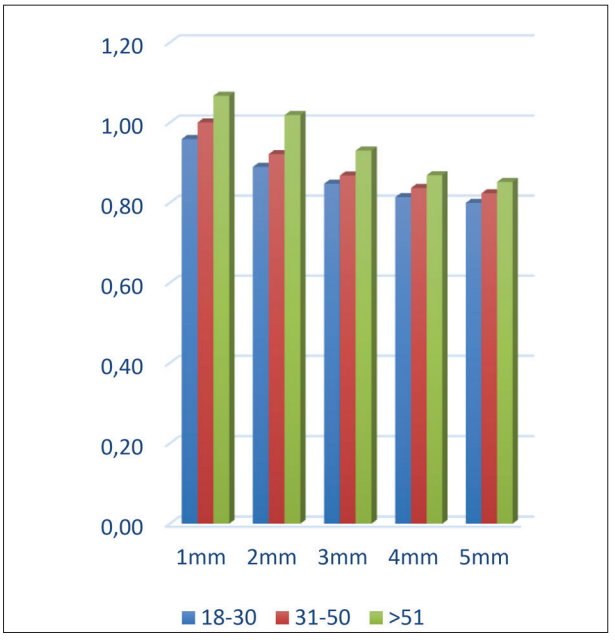

Figure 3. Dentin thickness ratio of MB root in relation to the measurement point in female population
Slika 3. Odnos debljine dentina meziobukalnog korena u odnosu na tačku merenja kod ženske populacije

It was also observed that there is a correlation between the thickness of the dentin below the furcation and the long / short root. The highest number of canal perforations was found in longer roots [11, 12]. Today, cone beam computerized tomography (CBCT) allows us to analyze the internal morphology of teeth in three dimensions.

The aim of this work was to check the average thickness of dentinal wall of the mesio-buccal root in first mandibular molar using CBCT axial sections of intact teeth.

MATERIAL AND METHODS

Based on a total of 372 examined CBCT images, 100 CBCT sections of teeth were selected, which were classified according to the following criteria: gender, age, length of the tooth and side of the jaw. Inclusion criteria of the teeth on which the measurement was performed:

1. CBCT images of the mandibular first molar (left or right)
2. Teeth without root fracture
3. Teeth without external and internal resorption
4. Teeth without root canal treatment
5. Teeth without calcification

According to gender, respondents were divided into the two groups (female and male), and according to age into the three age groups: 18–30 years, 31–50 years and older than 50 years. In relation to the side of the jaw, a division was made into the teeth of the left and right side. According to root length, teeth are classified into the three groups measured from the root furcation into: short root (7–9 mm), medium-length root (9–11 mm) and long root (over 11 mm). The distal wall of the mesiobuccal and mesiolingual root canals was designated as the risk zone of the mesial root of the lower first molar. The reason for this is the canal morphology of the tooth itself, as well as the infectious processes that occur in the pulpo-dentinal complex during life and that affects dentin thickness in the risk zones. Axial sections on CBCT images were used to measure dentin thickness. Dentin thickness was measured at the distance from the root furcation in the amount of: 1 mm, 2 mm, 3 mm, 4 mm and 5 mm (Figure 1).

RESULTS

The relationship between the side of the jaw and the distance from the root furcation shows that there is no

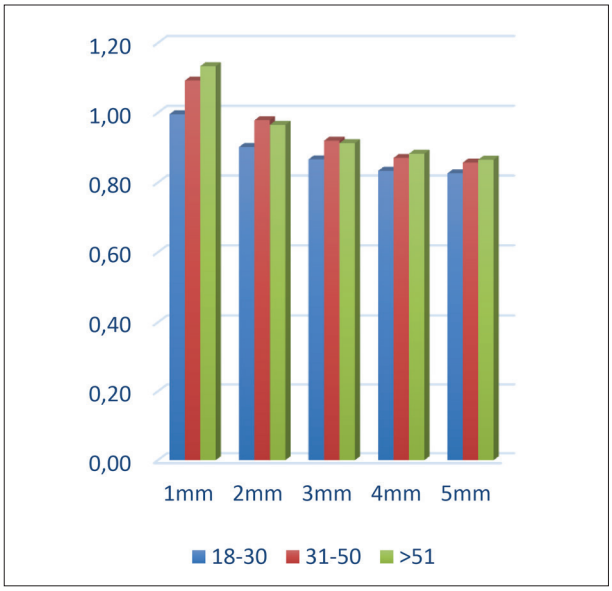

Figure 4. Dentin thickness ratio of ML root in relation to the measurement point in male population
Slika 4. Odnos debljine dentina meziolingvalnog korena u odnosu na tačku merenja kod muške populacije

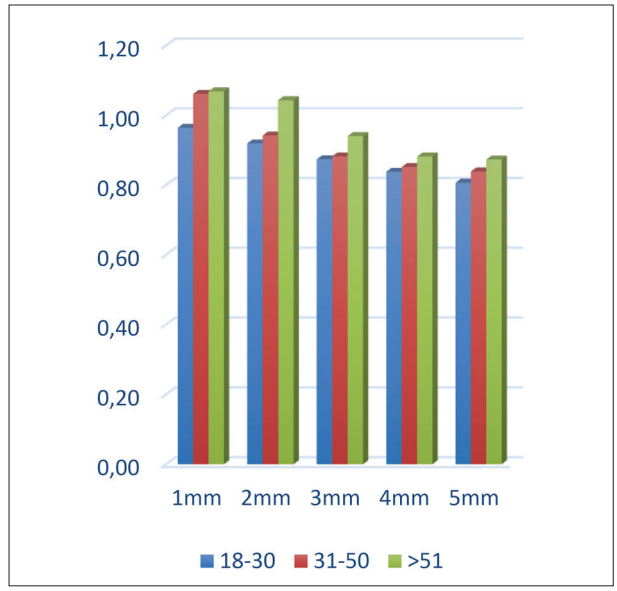

Figure 5. Dentin thickness ratio of ML root in relation to the measurement point in female population
Slika 5. Odnos debljine dentina meziolingvalnog korena u odnosu na tačku merenja kod ženske populacije

Table 1. Dentin thickness in teeth at different lengths in short, medium and long roots in both genders
Tabela 1. Debljina dentina na različitoj razdaljini u odnosu na dužinu korena (kratki, srednji, dugi) kod oba pola

ROOT LENGTH DUŽINA KORENA	Mesiobuccal root – male population Meziobukalni koren – muška populacija				
	1 mm	2 mm	3 mm	4 mm	5 mm
SHORT KRATKI	1.03 ± 0.10	0.91 ± 0.06	0.86 ± 0.03	0.84 ± 0.03	0.83 ± 0.03
MEDIUM SREDNJI	1.05 ± 0.08	0.95 ± 0.08	0.89 ± 0.07	0.86 ± 0.05	0.84 ± 0.05
LONG DUGI	0.97 ± 0.06	0.91 ± 0.06	0.86 ± 0.05	0.84 ± 0.03	0.84 ± 0.03
ROOT LENGTH DUŽINA KORENA	Mesiolingual root – male population Meziolingvalni koren – muška populacija				
	1 mm	2 mm	3 mm	4 mm	5 mm
SHORT KRATKI	1.06 ± 0.11	0.95 ± 0.06	0.90 ± 0.04	0.85 ± 0.03	0.85 ± 0.03
MEDIUM SREDNJI	1.11 ± 0.11	0.97 ± 0.09	0.91 ± 0.08	0.87 ± 0.06	0.86 ± 0.05
LONG DUGI	0.99 ± 0.08	0.92 ± 0.08	0.88 ± 0.04	0.86 ± 0.03	0.84 ± 0.03
ROOT LENGTH DUŽINA KORENA	Mesiobuccal root– female population Meziobukalni koren – ženska populacija				
	1 mm	2 mm	3 mm	4 mm	5 mm
SHORT KRATKI	1.00 ± 0.07	0.92 ± 0.07	0.87 ± 0.05	0.84 ± 0.04	0.83 ± 0.04
MEDIUM SREDNJI	1.00 ± 0.10	0.94 ± 0.08	0.88 ± 0.06	0.83 ± 0.03	0.81 ± 0.02
LONG DUGI	1.00 ± 0.00	0.92 ± 0.00	0.85 ± 0.00	0.82 ± 0.00	0.82 ± 0.00
ROOT LENGTH DUŽINA KORENA	Mesiolingual root – female population Meziolingvalni koren – ženska populacija				
	1 mm	2 mm	3 mm	4 mm	5 mm
SHORT KRATKI	1.01 ± 0.09	0.94 ± 0.07	0.88 ± 0.04	0.85 ± 0.03	0.84 ± 0.04
MEDIUM SREDNJI	1.03 ± 0.10	0.95 ± 0.08	0.88 ± 0.05	0.85 ± 0.04	0.83 ± 0.04
LONG DUGI	1.03 ± 0.00	0.95 ± 0.00	0.89 ± 0.00	0.85 ± 0.00	0.82 ± 0.00

statistically significant difference in dentin thickness in both male and female populations. This refers to mesiobuccal or mesiolingual canal in the mesial root ($p>0.05$). When it comes to the relationship between the length of the root and the distance from the furcation, there are differences in dentin thickness greater than 0.05 mm in both genders, as well as in both mesial canals, with the difference being more pronounced in male population. In the mesiobuccal root, it is observed that the difference in dentin thickness is most pronounced at 1, 2 and 3 millimeters of the canal and that it is the largest in the group of patients over 51 years of age, regardless of gender ($p < 0.05$) (Figures 2, 3).

Deviations in the thickness of dentin in different genders of mesiolingual root are statistically significant and most pronounced at 2 mm from the furcation of the roots ($p < 0.5$). Differences in thickness are more pronounced at 1 mm and 3 mm than at 4 mm and 5 mm of root furcation, but without statistical significance ($p > 0.5$) (Figures 4, 5).

At a distance of 1 and 2 mm from furcation, the thickness of dentin is greater in medium and short roots, and as the distance increases, these values increase in favor of long roots and thus equalize. This difference is more pronounced in mesiolingual root than in mesiobuccal, but without statistical significance, which is attributed to the morphology of the root itself and the process of dentinogenesis ($p = 0$) (Table 1).

When it comes to the relationship between age and distance from furcation, dentin thickness consistently increases with age. It is believed that sclerotic changes occurring in the

pulpo-dentinal complex are responsible for it. Primarily, there is a reduction of pulp chamber itself, which reduces its volume, so this is one of the reasons why endodontic treatment in elderly is difficult due to narrow and calcified canals. In older people, pulp chamber decreases due to reduced blood supply and formation of secondary physiological dentin, created more and more as the number of years increases, while in younger people, the pulp chamber is more voluminous. These changes occur equally in both genders and in both canals of the mesial root of the lower first molar ($p < 0.05$).

DISCUSSION

CBCT (Cone Beam Computerized Tomography) is based on cone beams, which are directed to a narrow area of interest and thus has a significantly reduced effective dose of radiation compared to conventional CT, high differentiation of details, accurate quantitative and qualitative values, economy and simplicity in the use of images. During life, pulpo-dentinal complex is changed by the physiological deposition of secondary dentin, which results in decrease of the size of the pulp chamber as well as the diameter of root canal. Consequently, in elderly, root canals are narrow and present a challenge to the clinician over time, while in younger individuals pulp chamber is more voluminous [7].

The risk zone of the tooth root represents the place of the smallest dentin thickness, which tends to decrease significantly during the formation of the tooth. Therefore, a better knowledge of the anatomy of the risk zone itself can reduce the risk of an adverse event.

In this paper, using CBCT imaging of one hundred lower first molars, measurements were made of the minimum thickness of the distal dentinal wall of mesiobuccal (MB) and mesiolingual (ML) canals at different distances from the furcation (1, 2, 3, 4 and 5 mm).

The results showed that in most cases the thickness of dentin in the risk zone was greater in male population compared to female population. As a result, women have a higher risk of unwanted perforation occurring during root canal treatment. It was also proven that minimal thicknesses of distal dentinal wall of MB and ML canals increased with age in each age group in both men and women at each measurement point.

When looking at the relationship between root length and furcation distance, in both genders there was a difference in dentin thickness greater than 0.05 mm for both mesial root canals, with this difference being more pronounced in men. When looking at the distance at 1 and 2 mm from furcation, dentin thickness is greater in short and medium roots, and by increasing distance, this value becomes equal to that of long roots. Due to morphology of the root, this difference is more pronounced in the mesiolingual canal. Some other studies have also shown that the thickness of the distal wall of the mesial root is smaller in longer teeth and therefore the risk of perforation is higher in teeth with longer roots [5, 6].

In the population of Banja Luka, the risk zone of dentin thickness is located at a distance of 2 to 3 mm from furcation with a range of 0.85 to 0.97 mm, while at a distance of 1 mm the value of dentin thickness is significantly higher 0.98 to 1.11 mm. It is precisely this zone that represents site exposed to canal perforation during endodontic treatment. Some studies found dentin thickness of 0.67 mm at a distance of 3 to 4 mm from furcation, which is much less than usually reported [4]. Another study conducted on 50 molars showed that the thinnest point of the risk zone is 3 mm from furcation with a mean thickness of 0.81 mm [1]. Similar results were obtained in some other studies [2, 5, 6, 9]. Some other authors such as Asgary et al. reported a decrease in average dentin thickness from 1.17 to 0.90 mm, confirming that the greatest decrease in dentin thickness occured at the level of the cervical third of the root [1].

Renato Menezes and his collaborators performed an examination by cutting 100 mesial roots 2 mm below the furcation and the thickness of distal dentinal wall was measured using microscope. The results obtained were that the average dentin thickness was 0.79 mm, and no significant differences were observed between mesiobuccal and mesiolingual canals [10].

Overinstrumentation of the root canal space can lead to perforation, according to Lim and Stock, a dentin thickness of 200 to 300 μm should be maintained after preparation to withstand forces during obturation and prevent perforation or vertical root fracture. During root canal treatment, instruments should be directed towards the lateral and mesial walls, which have much thicker dentin, and in the risk zone, the dentin should be thinned no more than 0.5 mm [7].

Vesal Feiz Azimi et al. state that the mean dentin thickness is 2 mm below furcation in mandibular molars from 0.78 to 1.27 mm. By comparing the thickness of dentin, they came to the conclusion that there are differences in the thickness of 2 mm below furcation between long and short roots [11]. The thinnest walls were measured on the distal walls of the longest roots, so caution is recommended when instrumenting the canal and avoiding the use of larger diameter canal instruments to avoid perforations. Analysis of root morphology by CBCT is necessary due to different root canal morphology and genetic conditioning. Today, it is known that ethnicity determines morphological characteristics, which provides a framework for endodontic therapy. Endodontic treatment involves mechanical use of instruments in combination with chemical agents. If during all these stages, we do not know the morphology of the root and dentin thickness, the appearance of unwanted complications is possible - mechanical, chemical or thermal nerve injuries that can cause neuropathic pain or anesthesia in its innervation zones.

Even 10% of the lower second molars have the possibility of injury to the lower alveolar nerve. To better inform the clinician, it is important to assess differences in root and canal shape among different racial populations and subpopulations [12, 13]. The prevalence of root fusion in first and second lower molars is 0.7% and 12.6%,

respectively, which can affect the thickness of the dentin in danger zone, i.e. the curvature of their mesial roots [14].

Also, research indicates an increase in diversity in the number and shape of root canals of mandibular molars. The most common morphological variation in lower molars is a supernumerary root located either lingually (radix entomolaris) or buccally (radix paramolaris) and occurs with varying frequency in all permanent mandibular molars (for Europeans – less than 4% frequency) [15, 16].

CONCLUSION

This research showed that the smallest thickness of dentinal walls of the risk zone is located 2 to 3 mm below furcation. Knowing this location leads to greater caution and a better plan during endodontic treatment to avoid unwanted consequences. You should also pay more attention to younger patients, especially if their teeth have longer roots.

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Received: 2.3.2023 • Accepted: 25.5.2023

Određivanje debljine dentina bukomezijalnog korena donjeg molara kompjuterizovanom konusnom tomografijom

Aleksandra Đeri[1], Irena Radman-Kuzmanović[1], Nikola Moravac[2], Milan Tejić[3], Renata Josipović[1], Adriana Arbutina[1], Saša Marin[1], Nataša Trtić[1], Valentina Veselinović[1]

[1]Univerzitet u Banjoj Luci, Medicinski fakultet, Odsek za dentalnu medicinu, Bosna i Hercegovina;
[2]ZU „Simić", Banja Luka, Bosna i Hercegovina;
[3]ZU „Zavod za stomatologiju", Banja Luka, Bosna i Hercegovina

KRATAK SADRŽAJ

Uvod Cilj ovog rada je bio da se proveri prosečna debljina distalnog zida mezijalnog korena prvog donjeg molara kod stanovnika Banjaluke primenom CBCT aksijalnih preseka intaktnih zuba.

Metode U ovoj studiji poprečnog preseka procenjeno je 100 CBCT slika prvih mandibularnih molara iz radiološkog centra Promax 3Dent u Banjoj Luci, u Bosni i Hercegovini. Korišćen je aparat Planmeca Promax3D Max (Planmeca, Helsinki, Finska), sa vidnim poljem (FOV) od 8 × 8 cm i rezolucijom od 0,1 mm. Analiza je vršena pomoću softvera Romexis Viewer verzije 3.1.1 (Planmeca, Helsinki, Finska). U aksijalnoj debljini 0,1 mm poprečni preseci sa razmakom od 1 mm su pravljeni ispod područja furkacije (pri povećanju od ×10) na rastojanju 1, 2, 3, 4, 5 mm od furkacije. Podaci su zatim analizirani uparenim t-testom.

Rezultati Debljina dentinskog zida u kanalu bukomezijalnog korena donjeg prvog molara bila je značajno manja na visini 2 mm i 3 mm nego merenjem na 1, 4 i 5 mm ($p < 0,05$).

Nije bilo značajne razlike između merenja na 1, 4 i 5 mm ispod područja furkacije ($p = 0,001$).

Zaključak S obzirom na malu debljinu dentinskog zida na 2-3 mm ispod furkacije u korenskom kanalu, potreban je oprez pri obradi bukomezijalnog kanala donjeg prvog molara i ispravan odabir tehnike preparacije kako ne bi došlo do transportacije kanala ili frakture korena.

Ključne reči: konusna kompjuterska tomografija; mandibularni molari; debljina dentina

UVOD

U ljudskoj denticiji, donji prvi molar je prvi iz grupe stalnih molara koji se pojavljuje u usnoj šupljini i to već u šestoj godini života. Rana pojava karijesa je razlog zbog kojeg je on jedan od češće tretiranih zuba u okviru restaurativnih te endodontskih tretmana. Na području dečije stomatologije donji prvi molar je zub na kojem se vrši zalivanje fisura. Sa tačke gledišta stomatološke protetike to je zub koji pripada grupi zuba koji su odgovorni za visinu zagrižaja, dok je u ortopediji vilica jedan od zuba koji se posmatra prilikom određivanja ortodontske nepravilnosti. Zbog navedenog, poznavanje morfologije donjeg prvog molara je od velike važnosti u stomatologiji. Prvi molari mandibule su tretirani endodontski, sa incidencom od 17% [1].

Prvi mandibularni molar ima složenu morfologiju, jedan mezijalni i jedan distalni koren. Mezijalni koreni mandibularnih molara obično imaju dva glavna kanala: meziobukalni i meziolingvalni. Specifičnost u anatomskoj građi donjih prvih molara ogleda se u većoj konkavnosti distalne površine bukomezijalnog korena. Debljina dentina na distalnoj površini bukomezijalnog korena donjeg prvog molara je ograničena, što treba imati u vidu pri endodontskom tretmanu kako bi se izbegla preinstrumentacija i fraktura korena zuba [2]. Područje furkacije molara prepoznato je kao jedno od najranjivijih područja. Kao zona rizika posebno se navodi i debljina dentina u distalnim zidovima mezijalnih kanala [3, 4, 5].

Pojava perforacija i preloma korena tokom endodontske terapije se češće javlja u ovim područjima, te dobro razumevanje unutrašnje anatomije može pomoći u prevenciji prekomernog gubitka dentinskog tkiva u opasnoj zoni, što može dovesti do preloma korena zuba pod funkcionalnim opterećenjem [6].

Ako kliničar zanemaruje debljinu zida dentina tokom procesa lečenja, suočiće se sa perforacijom zida kanala korena zuba. Perforacija se javlja posebno u zakrivljenim kanalima korena gde je kanal nepažljivo obrađivan kanalnim instrumentima većeg promera [7].

Mezijalni koren karakteriše široka bukolingvalna i uska meziodistalna dimenzija, dok je distalni koren uglavnom koničan. Morfologija svakog zuba je individualna i može biti povezana sa određenim etničkim grupama. Utvrđeno je da se opasna zona kod kineske i indijske populacije nalazi 4 do 6 mm ispod ulaza u kanal korena zuba, a minimalna debljina distalnog dentina nalazila se između 1 i 2 mm ispod furkacije [8, 9, 10].

Takođe je uočeno da postoji korelacija između debljine dentina ispod furkacije i dugih/kratkih korenskih zuba. Najveći broj perforacija korenskih kanala je pronađen u najdužim zubima [11, 12]. Danas nam kompjuterizovana tomografija sa konusnim snopom (CBCT) omogućava trodimenzijalnu analizu unutrašnje morfologije zuba.

Cilj ovog rada je bio da se proveri prosečna debljina distalnog zida mezijalnog korena prvog donjeg molara kod stanovnika Banjaluke primenom CBCT aksijalnih preseka intaktnih zuba.

MATERIJAL I METODE

Na osnovu ukupno pregledana 372 CBCT snimka izdvojeno je 100 CBCT preseka zuba koji su svrstani prema sledećim kriterijumima: pol ispitanika, životna dob ispitanika, dužina korena zuba i strana vilice. Inkluzioni kriterijumi zuba na kojima je izvršeno merenje bili su:

1. CBCT slike prvog molara mandibule (leva ili desna)
2. zubi bez preloma korena
3. zubi bez spoljne i unutrašnje resorpcije
4. zubi bez tretmana korenskog kanala
5. zubi bez kalcifikacije

Prema polu ispitanici su podeljeni na ženski i muški pol, a prema životnoj dobi u tri starosne grupe: 18–30 godina, 31–50 godina i stariji od 50 godina. U odnosu na stranu vilice, izvršena je podela na zube leve i desne strane. Prema dužini korena, zubi su klasifikovani u tri grupe prema udaljenosti od furkacije korena: kratki koren (7–9 mm), koren srednje dužine (9–11 mm) i dugi koren (preko 11 mm). Kao rizična zona mezijalnog korena donjeg prvog molara označen je distalni zid meziobukalnog i meziolingvalnog kanala korena zuba. Razlog za to je kako sama kanalna morfologija zuba, tako i infektivni procesi koji se dešavaju u pulpo-dentinskom kompleksu tokom života i koji utiču na debljinu dentina u rizičnim zonama. Za merenje debljine dentina korišćeni su aksijalni preseci na CBCT snimcima.

Merena je debljina dentina na udaljenosti od furkacije korena u iznosu od: 1 mm, 2 mm, 3 mm, 4 mm i 5 mm (Slika 1).

REZULTATI

Odnos između strane vilice kojoj pripada zub i udaljenost od furkacije korena pokazuje da ne postoji statistički značajna razlika u debljini dentina kako kod muške tako i kod ženske populacije. Ovo se odnosi na meziobukalni, odnosno meziolingvalni kanal u mezijalnom korenu, $p > 0,05$. Kada je u pitanju odnos između dužine korena i udaljenosti od furkacije, postoje razlike u debljini dentina veće od 0,05 mm kod oba pola, kao i kod oba kanala mezijalnog korena, s tim što je kod muške populacije ta razlika izraženija. Kod bukomezijalnog korena se uočava da je razlika u debljini dentina najizraženija na 1, 2. i 3. milimetru kanala korena zuba i da je najveća u grupi pacijenata preko 51 godine života bez obzira na pol ($p < 0,05$) (Grafikoni 1 i 2).

Odstupanja u debljini dentina kod različitih polova lingvomezijalnog korena su statistički značajna i najviše izražena na 2 mm od furkacije korenova ($p < 0,5$). Razlike u debljini su izraženije na 1 mm i 3 mm nego na 4 mm i 5 mm od furkacije korenova, ali bez statističke značajnosti ($p > 0,5$) (Grafikoni 3 i 4).

Na udaljenosti 1 i 2 mm od furkacije debljina dentina je veća kod srednjih i kratkih korenova da bi se povećanjem udaljenosti te vrednosti povećale u korist dugih korenova i na taj način izjednačile. Ova razlika je izraženija u meziolingvalnom korenu nego u meziobukalnom, ali bez statističke značajnosti, što se pripisuje samoj morfologiji korena i procesu dentinogeneze ($p = 0$) (Tabela 1).

Kada je u pitanju povezanost između starosne dobi i udaljenosti od furkacije, debljina dentina se konstantno povećava sa povećanjem broja godina. Smatra se da su za to odgovorne sklerotične promene koje se dešavaju u pulpo-dentinskom kompleksu. Prvenstveno dolazi do smanjenja same pulpne komore, koja smanjuje svoj volumen, pa je i to jedan od razloga zbog kojih je endodontska terapija kod starijih osoba otežana usled uskih i neprohodnih kanala. Kod starijih osoba se pulpna komora smanjuje zbog smanjene prokrvljenosti, a i zbog formiranja sekundarnog fiziološkog dentina, koji se sve više stvara kako se povećava broj godina, dok je kod mlađih osoba pulpna komora voluminoznija. Ove promene se podjednako dešavaju kod pripadnika oba pola i kod oba kanala mezijalnog korena donjeg prvog molara. Uočava se statistički značajna razlika u debljini dentina između prve i treće starosne grupe na svim mernim tačkama, koja iznosi između 0,04 mm i 0,06 mm ($p < 0,05$).

DISKUSIJA

Kompjuterizovana tomografija sa konusnim snopom temeljena je na konusim zracima, koji su usmereni na usko područje interesa, te tako ima bitno smanjenu efektivnu dozu zračenja u odnosu na konvencionalnu kompjuterizovanu tomografiju, visoku diferencijaciju detalja, tačne kvantitativne i kvalitativne vrednosti, ekonomičnost i jednostavnost u korišćenju snimaka. Tokom života pulpo-dentinski kompleks se menja fiziološkim taloženjem sekundarnog dentina, što za posledicu ima smanjenje veličine pulpne komore kao i promera korenskog kanala. Posledično, kod starijih osoba su kanali korena zuba uski i tokom vremena predstavljaju izazov za kliničara, dok je kod mlađih osoba pulpna komora voluminoznija [7].

Rizična zona korena zuba predstavlja mesto najmanje debljine dentina, koja ima tendenciju značajnog smanjenja tokom oblikovanja kanala korena zuba. Zbog toga, bolje poznavanje same anatomije rizične zone može smanjiti rizik od neželjenog događaja.

U ovom radu su, korišćenjem CBCT snimanja stotinu donjih prvih molara, vršena merenja najmanje debljine distalnog dentina meziobukalnih i meziolingvalnih kanala na različitim udaljenostima u odnosu na furkaciju (1, 2, 3, 4 i 5 mm).

Rezultati su pokazali da je u većini slučajeva debljina dentina rizične zone veća kod muške populacije u odnosu na žensku populaciju. Samim tim je kod žena veći rizik da se dogodi neželjena perforacija tokom obrade kanala korena zuba. Takođe dokazano je da se minimalne debljine distalnog dentina meziobukalnih i meziolingvalnih kanala povećavaju s godinama u svakoj starosnoj skupini i kod muškaraca i kod žena na svakoj mernoj tački.

Kada se posmatra odnos između dužine korena i udaljenosti od furkacije, kod oba pola postoji razlika u debljini dentina koja je veća od 0,05 mm za oba kanala mezijalnog korena, s tim što je ta razlika izraženija kod muškaraca. Kada se gleda udaljenost na 1 i 2 mm od furkacije, debljina dentina je veća kod kratkih i srednjih korenova da bi se povećanjem udaljenosti ta vrednost izjednačila sa dugim korenovima. Zbog same morfologije korena ova razlika je značajnije izražena u meziolingvalnom kanalu. Neke druge studije su takođe pokazale da je debljina distalnog zida mezijalnog korena manja kod dužih zuba, te je samim tim veći rizik od perforacije kod zuba sa dužim korenom [5, 6].

Kod populacije u Banjaluci rizična zona debljine dentina se nalazi na 2 do 3 mm udaljenosti od furkacije sa rasponom od 0,85 do 0,97 mm, dok je na udaljenosti od 1 mm vrednost debljine dentina znatno veća – 0,98 do 1,11 mm. Upravo ova zona predstavlja predilekciono mesto za nastanak perforacije kanala prilikom endodontskog tretmana.

Vredi napomenuti da su neke studije dolazile do rezultata da je debljina dentina 0,67 mm na udaljenosti 3 do 4 mm od furkacije, što je dosta manje nego što se obično navodi [4].

Druga studija, koja je rađena na 50 molara, pokazala je da je najtanja tačka rizične zone na 3 mm od furkacije sa srednjom debljinom od 0,81 mm [1]. Slični rezultati su dobijeni i u nekim drugim studijama [2, 5, 6, 9]. U ovoj studiji se navodi da su neki drugi autori, kao što su Asgary i saradnici, prijavili smanjenje prosečne debljine dentina sa 1,17 na 0,90 mm, potvrđujući da se najveće smanjenje debljine dentina događa na nivou cervikalne trećine korena [1].

Renato Menezes sa saradnicima je vršio ispitivanje tako što je 100 mezijalnih korenova prerezano 2 mm ispod furkacije i nakon toga je uz pomoć mikroskopa vršeno merenje debljine distalnog dela dentina. Dobijeni su rezultati da je prosečna debljina dentina iznosila 0,79 mm, te da nisu uočene značajne razlike između meziobukalnih i meziolingvalnih kanala [10].

Prekomerno proširenje prostora kanala korena može dovesti do perforacije. Lim i Stock smatraju da nakon preparacije treba zadržati debljinu dentina od 200 do 300 μm kako bi se izdržale sile tokom opturacije i sprečila perforacija ili vertikalna fraktura korena. Tokom obrade kanala korena instrumente treba usmeravati prema bočnim i mezijalnim zidovima koji imaju mnogo deblji dentin, a u rizičnoj zoni dentin stanjiti ne više od 0,5 mm [7].

Vesal Feiz Azimi i saradnici navode da je srednja debljina dentina 2 mm ispod furkacije

kod mandibularnih molara od 0,78 do 1,27 mm. Poređenjem debljine dentina došli su do zaključka da postoje razlike u debljini 2 mm ispod furkacije između dugih i kratkih korenova [11]. Najtanji zidovi su izmereni na distalnim zidovima najdužih korenova, pa se preporučuje oprez kod obrade kanala i izbegavanje upotrebe kanalnih instrumenata većeg promera kako bi se izbegle perforacije. Analiza korenske morfologije CBCT-om je neophodna zbog individualnosti morfologije korenskih kanala i genetske uslovljenosti. Danas se zna da etnička pripadnost određuje morfološke karakteristike, što daje okvir za endodontsku terapiju. Endodontski tretman uključuje mehaničku upotrebu instrumenata u kombinaciji sa hemijskim sredstvima. Ukoliko tokom svih ovih faza ne poznajemo morfologiju korena zuba i debljinu dentina, moguća je pojava neželjenih komplikacija – mehaničkih, hemijskih ili termičkih povreda nerva koje mogu uzrokovati neuropatski bol ili anesteziju u svojoj inervaciji zona. Tokom endodontskog tretmana 1% donjih pretkutnjaka pa čak i 10% donjih drugih kutnjaka postoji mogućnost ozlede donjeg alveolarnog živca. Za bolje informisanje kliničara je važno proceniti razlike u obliku korena i kanala među različitim rasnim populacijama i subpopulacijama [12, 13].

Treba imati u vidu da je za prvi i drugi donji kutnjak prevalencija fuzije korenova 0,7%, odnosno 12,6%, što može uticati na debljinu dentina u opasnoj zoni odnosno krivini mezijalnog korena prvog i drugog donjeg molara [14].

Takođe, odontološka istraživanja ukazuju na povećanje raznolikosti u broju i obliku korenskih kanala mandibularnih kutnjaka. Najčešća morfološka varijacija kod donjih molara je prekobrojan koren koji se nalazi ili lingvalno (radix entomolaris) ili bukalno (radix paramolaris) i javlja se s različitom učestalošću kod svih trajnih mandibularnih kutnjaka (kod Evropljana sa manje od 4% učestalosti) [15, 16].

ZAKLJUČAK

Ovo istraživanje je pokazalo da se najmanja debljina zidova dentina rizične zone nalazi na 2 do 3 mm ispod furkacije. Poznavanje ove lokacije navodi na veći oprez i bolji plan tokom endodontske terapije kako bi se izbegle neželjene posledice. Takođe treba obratiti veću pažnju kod mlađih pacijenata, pogotovo ako njihovi zubi imaju duže korenove.

ORIGINAL ARTICLE
ORIGINALNI RAD
https://doi.org/10.2298/SGS2302079J
UDC: 615.46:616.314

Marginal microleakage of newly synthesized nanostructured materials based on calcium aluminate after application in interradicular perforations

Renata Josipović[1], Violeta Petrović[2], Adriana Arbutina[3], Irena Kuzmanović Radman[1], Aleksandra Đeri[1], Nataša Gajić[1], Radmila Arbutina[1], Slavoljub Živković[2]

[1]University of Banja Luka, Faculty of Medicine, Department of Dental Diseases, Banja Luka, Republika Srpska, Bosnia and Herzegovina;
[2]University of Belgrade, Faculty of Dentistry, Clinic for Dental Diseases, Belgrade, Serbia;
[3]University of Banja Luka, Faculty of Medicine, Department of Jaw Orthopedics, Banja Luka, Republika Srpska, Bosnia and Herzegovina

SUMMARY

Introduction Marginal seal or adequate marginal adaptation of material along the cavity walls should be able to prevent leakage of tissue fluid and consequently bacterial microleakage, terefore, it is considered as significant factor for long term success of endodontic treatment. The aim of this work was to test the marginal microleakage of newly synthesized nanostructured biomaterials based on calcium aluminate, calcium silicate and MTA with a dye penetration test after application the material to the interradicular perforations of extracted teeth.

Material and method The study included 48 extracted human maxillary and mandibular molars. Newly synthesized nanostructured materials were tested: material based on calcium aluminate, calcium silicate. Commercial calcium silicate cement (MTA Angelus, Londrina, Brazil) was used as a control material. Marginal microleakage was examined with a dye penetration test six months after application of the material in experimentally prepared interradicular perforations on extracted human molars. Penetration depth measurement was analyzed with a binocular magnifier (Leica DM 500, Leica Byosistems). Results are expressed in millimeters and statistically processed by applying the analysis of variance for repeated measurements with the Sidak test.

Results The lowest average penetration (mm) was for MTA (1.40 ± 0.63 mm), and the highest for Ca aluminates (2.10 ± 0.63 mm), while for calcium silicates an average color penetration of 1.73 ± 0, 67mm was recorded. By testing intergroup differences in color penetration between groups, a statistically significant difference between MTA and Ca aluminate was obtained using Sidak's test (t = -0.693; p = 0.036). No statistically significant difference was recorded between MTA and Ca silicate, nor was there a statistically significant difference between Ca aluminate and Ca silicate.

Conclusion The lowest marginal microleakage, the best marginal sealing, was recorded with the MTA material. The microleakage of calcium aluminate-based materials was significantly higher compared to calcium silicate and MTA.

Keywords: marginal micropermeability; calcium aluminates; calcium silicates

INTRODUCTION

Perforation means mechanical or pathological communication between the root canal system and the outer surface of the tooth caused by caries, resorption or iatrogenic factors [1]. According to localization, root perforations can be interradicular, lateral and apical. Untreated perforations cause inflammation, resorption of bone, dentin and cementum. This is especially pronounced in perforations in the furcation of multi-rooted teeth. The main goal in the treatment of perforations is to prevent inflammatory process and consequent loss of tissue attachment at the site of the perforation, i.e. the establishment of a new tissue attachment, if the lesion already exists [2].

Many materials have been used in the therapy of root canal perforations: zinc oxide-eugenol, amalgam, calcium hydroxide, glas ionomer, cavity, gutta-percha, composite materials, hydroxyapatite, calcium phosphate cements, tricalcium phosphate cements, IRM (Intermediate Restorative Material), glas ionomer cements, Portland cement, MTA. But none of them completely satisfy all the requirements [3, 4].

The properties that an ideal material for closing perforations should have are: biocompatibility, short bonding time, ability to have a good edge closure, as well as an appropriate antibacterial effect, that it is insoluble in tissue fluids and that it has adequate mechanical properties. The material of choice in the treatment of root perforations is MTA, presented as early as 1993 by Loma Linda University [5, 6].

MTA has good physical, chemical and biological characteristics, and one of its indications is root canal

Address for correspondence: Renata JOSIPOVIĆ, University of Banja Luka, Faculty of Medicine, Department of Dental Diseases, Banja Luka, Republika Srpska, Bosnia and Herzegovina; *renata.josipovic@med.unibl.org*

perforations. Due to its antimicrobial effect and very high pH, it enables the regeneration of periodontal ligaments around the site of injury, perforation. In their study, Main et al. concluded that MTA provides effective marginal closure in root canal perforations. Placement of the material in an appropriate period of time can prevent bacterial contamination from the pulp chamber into the periodontal tissue [7]. However, the time it takes to bind MTA is 2 hours and 30 minutes. The ability to seal the edge of MTA in an aqueous environment may be compromised during the first 72 hours due to the solubility of the material. Mohan et al. claimed that it takes 3 days for MTAs to show good marginal closure.

Contemporary research is focused on the synthesis and testing of the possible application of nanostructured biomaterials in endodontic indications. In recent years, at the Institute for Nuclear Research in Vinča, according to the recipe of V. Jokanović, a new nanostructured material based on the calcium-aluminate system obtained by the hydrothermal sol-gel method and a self-propagating combustion reaction was synthesized. This method of synthesis provides high activity of particles, faster hydration and short bonding time [9]. Calcium aluminate-based cements provide great potential in the field of biomaterials due to the reduced setting time and associated microstructure.

The aim of this work was to test the marginal microleakage of newly synthesized nanostructured biomaterials based on calcium aluminate, calcium silicate and MTA with a dye penetration test after applying the material to the interradicular perforations of extracted teeth.

MATERIAL AND METHOD

In this research, materials based on calcium aluminate systems (CA), white Mineral Trioxide Aggregate MTA (White MTA, Angelus® Soluções odontológicas Londrina, Brazil) and materials based on calcium silicate (CS) were tested. The study included 48 extracted human maxillary and mandibular molars with fully developed roots. The access cavities were prepared with a high-speed handpiece, after which the working length of the root canal was determined with hand files (Kfiles, VDW Gmbh, Germany). The working length was determined up to the physiological foramen, 1 mm shorter than the anatomical foramen. All root canals were prepared using Crown-Down technique. During the preparation of the root canal, copious irrigation with 0.5% NaOCl was performed. The canals were dried with paper points and obturated using the monocone technique with sealer AH PlusTM (Dentsply, Germany).

After incubation of the samples, perforation of the floor of pulp chamber was performed between the roots (interradicular perforation). Perforation was done with a high speed handpiece and a size #4 round drill bit. The width of the perforations corresponded to the diameter of the burr, while the depth depended on the thickness of the floor of the pulp chamber. Interradicular perforations were washed with distilled water and divided into the three groups, after which they were closed with the tested materials.

Group 1: calcium silicate was used to close the perforations - 12 samples.

Group 2: MTA was used to close the perforations - 12 samples.

Group 3: calcium aluminate-based material was used to close the perforations - 24 samples.

Before the application of tested materials, the teeth were placed in a sponge soaked in distilled and deionized water up to the level of the enamel-cementum border. The calcium aluminate-based material was mixed with distilled water in a ratio of 3:1 and control materials were mixed according to the manufacturer's instructions, then placed in the furcal perforations with the help of a reamer. Wet cotton pellet was placed over the material, and then the teeth were incubated at 37°C for 24 h. After bonding the material, the access cavities were definitely closed with the composite. The teeth were stored in a sponge soaked with distilled and deionized water and incubated at 37°C for the next 6 months.

After 6 months, the marginal microleakage was measured. The teeth were coated with two layers of nail polish, except in the area around furcation (1 mm around the furcation). After that, all samples were immersed in a 50% solution of silver nitrate (AgNO3) for 2 h, and then washed in photographic developer for the next 6 h. Then longitudinal sectioning of the teeth was performed through the perforation itself with a 0.7 mm thick diamond disc, linear precision saw with water cooling (Isomet saw 4000, Buehler, Lake Bluff, IL, USA). Penetration depth was measured with a binocular magnifier (Leica DM 500, Leica Byosistems). The obtained values were expressed in millimeters, and results were statistically analyzed using analysis of variance for repeated measurements with the Sidak test.

RESULTS

The lowest average penetration (mm) was for MTA (1.40 ± 0.63mm), and the highest for Ca aluminates (2.10 ± 0.63mm), while for calcium silicates an average color penetration of 1.73 ± 0.67 mm was recorded.

By testing intergroup differences in color penetration between groups, a statistically significant difference between MTA and Ca aluminate was obtained using Sidak's test ($t=-0.693$; $p=0.036$). No statistically significant difference was recorded between MTA and Ca silicate, nor was there a statistically significant difference between Ca aluminate and Ca silicate.

DISCUSSION

Many methods of dye penetration [10], liquid filtration [11], bacterial penetration [12] and protein penetration [13] have been used to investigate microleakage.

In this research, linear dye penetration method was used to assess microleakage. This method was also most commonly used method for evaluating the quality of edge closure due to its simplicity of application. Despite the

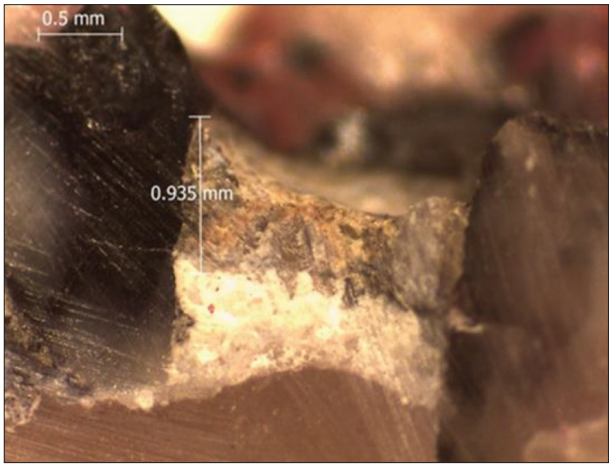

Figure 1. Inter-radicular perforation filled with material based on calcium aluminate. There is partial dissolution of the material, that is, disintegration with absorption of color and partial color change of the material (×30).
Slika 1. Interardiksna perforacija ispunjena materijalom na bazi kalcijum-aluminata. Uočava se delimično rastvaranje materijala, odnosno dezintegracija sa apsorpcijom boje i delimičnim prebojavanjem materijala (30×).

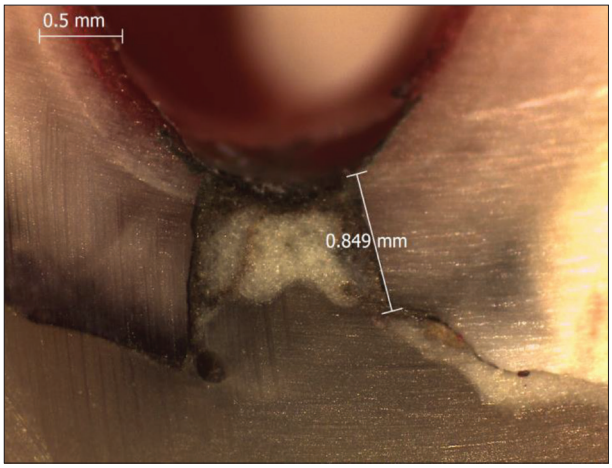

Figure 2. Inter-radicular perforation filled with MTA. There is dye absorption with most of the material having changed color (×30).
Slika 2. Interradiksna perforacija ispunjena sa MTA. Uočava se marginalni prodor boje sa prebojavanjem većeg dela materijala (30×).

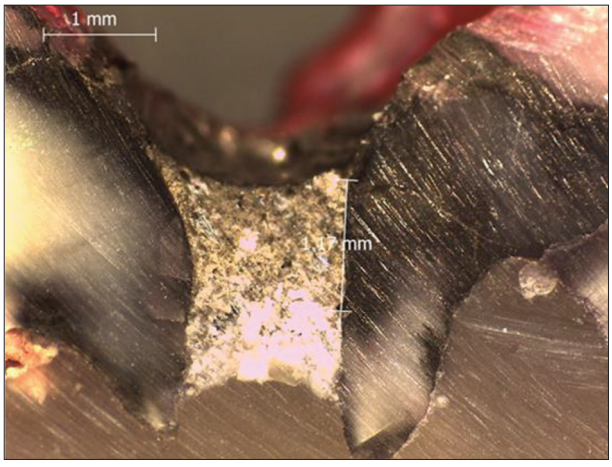

Figure 3. Perforation filled with calcium silicate material. There is marginal dye leakage with color change of material (×30).
Slika 3. Perforacija ispunjena materijalom kalcijum-silikatom. Uočava se izražen prodor boje sa prebojavanjem dela materijala (30×).

Table 1. Mean values and standard deviations of color penetration for the tested materials
Tabela 1. Srednje vrednosti i standardne devijacije prodora boje ispitivanih materijala

Material N	$\bar{x}$	SD	Med	Min	Max
Ca aluminates Ca-aluminati 24	2.10	0.63	2.19	0.83	3.25
Ca silicates Ca-silikati 12	1.73	0.67	1.78	0.00	2.97
MTA 12	1.40	0.63	1.47	0.00	2.24
Total Ukupno 48	1.74	0.68	1.76	0.00	3.25

mentioned advantages, dye penetration method has several disadvantages: molecular size of most dye particles is larger than the size of bacteria, and the dye penetration is observed in one plane, i.e. point [14]. Dye penetration method is static, it does not reflect the dynamic interaction with the periradicular tissue, and it is not possible to determine the volume of dye between hard tooth tissue and dental materials. In the analysis of marginal closure of dental materials, many types of dyes were used (methylene blue, silver nitrate, basic fuchsin, rhodamine B...). Methylene blue is a commonly used dye due to its affordable price [15]. Silver nitrate solution was used in this research due to its stability in materials with high pH values. Silver nitrate particles possess a large molecular mass similar to the size of bacteria. Silver nitrate is not a hydrophilic solution, like basic fuchsin [16].

Torabinejad et al. indicated that a material capable of preventing the penetration of small dye molecules could also prevent the passage of bacteria and their by-products [17].

An ideal material for closing interradicular perforations should have dimensional stability, be easy to place, non-toxic, non-carcinogenic, have X-ray contrast and be biocompatible [18], as well as the ability to induce osteogenesis and cementogenesis [19].

In 1993, Torabinejad presented MTA as a biomaterial, which can lead to deposition of cement when used in the treatement of interradicular perforations [17]. This material has superior marginal sealing capabilities compared to other restorative materials in the indication of tooth root perforations. [20]. Aldayri et al. found that MTA, when placed in interradicular perforations, can induce the formation of cementum tissue [21].

In 2009, Jacobovitz M et al. analyzed bacterial microleakage of MTA and an experimental material based on calcium aluminate (EndoBinder). Both materials showed effective microleaking of *Enterococcus faecalis in vitro* after 30 days. The authors attribute this result to the addition of a dispersant, that is, additive that enables the reduction of the water content when mixing the material, which results in a denser structure [22].

In this research, linear penetration of the dye was measured, expressed in millimeters, to the point that dye penetrated after longitudinal sectioning of the tooth. The

disadvantage of this method is that dye penetration could not be determined at the deepest point of penetration. The diameter of the experimental preparations corresponded to the diameter of the drill (size #4), while the depth, that is, the dimension of the perforation, depended on the thickness of the floor of pulp chamber. Camps and Pashley found that dye penetration relies on randomly cutting the root in two without knowing whether the section goes through the deepest dye penetration [23].

The results of our research showed that best marginal seal was achieved in interradicular perforations filled with MTA. Color penetration values for calcium silicate were slightly higher, but without a statistically significant difference. The highest color penetration was recorded in furcations that were filled with calcium aluminate cements. The obtained results of marginal micropermeability correspond to the results of solubility and porosity of the tested materials. The reason for such results may be the structure of the cement itself, built of numerous pores and capillaries that could lead to a certain permeability. Sarkar et al. in 2005 attributed good marginal seal of MTA to its ability to form hydroxyapatite and its deposition on the surface of the material in contact with tissue [24]. Jeffries et al. detected the ability to form surface apatite with cements based on calcium aluminate when closing marginal microcracks in simulated physiological conditions [25]. According to the results of previous research, MTA showed better marginal closure, less color penetration compared to other tested dental materials, which is in accordance with the results of our study [26]. This may be due to different consistency of dental materials. After mixing, MTA has a paste consistency, while when mixing calcium aluminate, it had a grainy consistency, which made it impossible to completely close the edges.

Pace et al. found that the use of biocompatible materials in the repair of furcal perforations leads to reduction in the inflammatory response of the surrounding tissues. With the use of MTA for furcal perforations in dog teeth, cement formation occurs over the material without inflammatory cell infiltrates. Therefore, MTA represents an ideal material in the treatment of furcal perforations, it is non-toxic and insoluble in a moist environment [27].

Comparing marginal sealing ability of MTA, calcium phosphate cement and bone cement in the repair of furcal perforations in an *in vitro* study on 70 human extracted molars, Chordiya et al. indicated the lowest micropermeability of MTA. They stated that good marginal sealing of MTA is a consequence of excellent adaptation of the material to the outer edges of the perforation due to the expansion of the material [28].

Examining bacterial microleakage of calcium aluminate-based materials in combination with glas ionomer cements, Pameijer et al. in an observation period of 60 days on 30 extracted premolars, showed that calcium aluminate-based material has good marginal closure and is bioactive, has the ability to form hydroxyapatite, providing the possibility of tissue remineralization. The authors attribute this result to the chemical composition of the material [29].

Haghgoo et al. analyzed microleakage in iatrogenic furcal perforations for MTA and calcium-enriched mixture. Color penetration was recorded with both materials, without statistically significant differences. The authors attribute this result to the hydrophilicity of both materials, that allowed good adaptation of the material to the perforation walls [15].

One of the possible reasons for good marginal seal of MTA is expansion of the material after setting, which increased the quality of seal. Another possibility is related to crystal deposition in MTA. In general, by reviewing the literature, it can be concluded that an ideal material that would prevent dye penetration, liquids, bacteria and microorganisms, still does not exist.

CONCLUSION

The lowest marginal micropermeability, i.e. the best marginal seal was recorded with MTA. Micropermeability of calcium aluminate-based materials was significantly higher compared to calcium silicate and MTA.

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Received: 10.3.2023 • Accepted: 5.6.2023

Ispitivanje marginalne mikropropustljivosti novosintetisanih nanostrukturnih materijala na bazi kalcijum-aluminata posle aplikacije u interradiksne perforacije

Renata Josipović[1], Violeta Petrović[2], Adriana Arbutina[3], Irena Kuzmanović Radman[1], Aleksandra Đeri[1], Nataša Gajić[1], Radmila Arbutina[1], Slavoljub Živković[2]

[1]Univerzitet u Banjoj Luci, Medicinski fakultet, Katedra za bolesti zuba, Banja Luka, Republika Srpska, Bosna i Hercegovina;
[2]Univerzitet u Beogradu, Stomatološki fakultet, Klinika za bolesti zuba, Beograd, Srbija;
[3]Univerzitet u Banjoj Luci, Medicinski fakultet, Katedra za ortopediju vilica, Banja Luka, Republika Srpska, Bosna i Hercegovina

KRATAK SADRŽAJ

Uvod Kvalitetno rubno zaptivanje, odnosno odgovarajuća marginalna adaptacija materijala uz zidove kaviteta, treba da onemogući protok tkivnih tečnosti i posledično bakterijsko mikrocurenje, zbog čega se smatra značajnim faktorom za dugoročan uspeh endodontskog lečenja. Cilj ovog rada bio je da se testom prodora boje ispita marginalna mikropropustljivost novosintetisanih nanostrukturnih biomaterijala na bazi kalcujum-aluminata, kalcijum-silikata i MTA a nakon primene materijala u interradiksne perforacije ekstrahovanih zuba.

Materijal i metode rada Ispitivanje je vršeno na 48 ekstrahovanih humanih maksilarnih i mandibularnih molara. Testirani su novosintetisani nanostrukturni materijali – materijal na bazi kalcijum-aluminata, kalcijum-silikata. Kao kontrolni materijal korišćen je komercijalni kalcijumsilikatni cement (MTA Angelus, Londrina, Brazil). Marginalna mikropropustljivost je ispitivana testom prodora boje šest meseci posle primene materijala u eksperimentalno preparisane interradiksne perforacije na ekstrahovanim humanim molarima. Merenje dubine prodora analizirano je binokularnom lupom (Leica DM 500, Leica Byosistems). Dobijene vrednosti su izražene u milimetrima, a dobijeni rezultati su statistički obrađeni primenjivanjem analize varijanse za ponovljena merenja Sidakovim testom.

Rezultati Najmanji prosečan prodor (mm) bio je za MTA (1,40 ± 0,63 mm), a najveći za kalcijum-aluminate (2,10 ± 0,63 mm), dok je za kalcijum-silikate zabeležen prosečan prodor boje od 1,73 ± 0,67 mm. Testiranjem međugrupnih razlika prodora boje između grupa Sidakovim testom dobijena je statistički značajna razlika između MTA i kalcijum-aluminata ($t = -0,693$; $p = 0,036$). Između MTA i kalcijum-silikata nije zabeležena statistički značajna razlika, kao ni između kalcijum-aluminata i kalcijum-silikata.

Zaključak Najmanja marginalna mikropropustljivost, odnosno najbolje rubno zaptivanje zabeleženo je kod materijala MTA. Mikropropustljivost materijala na bazi kalcijum-aluminata bila je značajno veća u odnosu na kalcijum-silikat i MTA.

Ključne reči: marginalna mikropropustljivost; kalcijum-aluminati, kalcijum-silikati

UVOD

Perforacija podrazumeva mehaničku ili patološku komunikaciju između sistema kanala korena i spoljašne površine zuba uzrokovanu karijesom, resorpcijom ili jatrogenim faktorima [1]. Prema lokalizaciji, perforacije korena mogu biti interradiksne, lateralne i apikalne. Nelečene perforacije uzrokuju inflamaciju, resorpciju kosti, dentina i cementa. Ovo je naročito izraženo kod perforacija u furkaciji višekorenih zuba. Glavni cilj u tretmanu perforacija je sprečavanje zapaljenskog procesa i posledičnog gubitka tkivnog pripoja na mestu perforacije, odnosno uspostavljanje novog tkivnog pripoja, ukoliko lezija već postoji [2].

Mnogi materijali su korišćeni u terapiji perforacija kanala korena: cink-oksid eugenol, amalgam, kalcijum-hidroksid, glas-jonomer, kavit, gutaperka, kompozitni materijali, hidroksiapatit, kalcijum-fosfatni cementi, trikalcijum-fosfatni cementi, IRM (Intermediate Restorative Material), glas-jonomerni cementi, Portland cement, MTA. Ali nijedan u potpunosti ne zadovoljava sve tražene zahteve [3, 4].

Osobine koje bi trebalo da poseduje idealni materijal za zatvaranje perforacija kanala korena zuba su: biokompatibilnost, kratko vreme vezivanja, sposobnost dobrog rubnog zatvaranja, kao i odgovarajući antibakterijski efekat, da je nerastvorljiv u tkivnim tečnostima i da poseduje adekvatna mehanička svojstva. Materijal izbora u terapiji korenskih perforacija je MTA, predstavljen još 1993. godine od strane Univerziteta Loma Linda [5, 6].

MTA poseduje dobre fizičke, hemijske i biološke karakteristike, te je jedna od njegovih indikacija i terapija perforacija kanala korena. S obzirom na antimikrobno dejstvo i veoma visok MTA, omogućava regeneraciju periodontalnih ligamenata oko mesta povrede, odnosno perforacije. Main i saradnici su u svojoj studiji zaključili da MTA obezbeđuje efektivno rubno zatvaranje kod perforacija kanala korena. Postavka materijala u odgovarajućem vremenskom periodu može da spreči bakterijsku kontaminaciju iz pulpne komore u periodontalno tkivo [7]. Međutim, vreme koje je potrebno za vezivanje MTA iznosi dva sata i 30 minuta. Sposobnost rubnog zatvaranja MTA u vodenoj sredini može biti ugroženo tokom prva 72 sata zbog rastvorljivosti materijala. Mohan i saradnici tvrde da je potrebno tri dana da MTA pokaže dobro rubno zatvaranje, kada se koristi kao materijal za zatvaranje kod perforacija kanala korena [8].

Savremena istraživanja su fokusirana na sintezu i ispitivanja moguće primene nanostrukturnih biomaterijala u endodontskim indikacijama. Poslednjih godina na Institutu za nuklearna istraživanja u Vinči prema recepturi V. Jokanovića sintetisan je novi nanostrukturni materijal na bazi kalcijum-aluminatnog sistema dobijen hidrotermalnom sol-gel metodom i samoširećom reakcijom sagorevanja. Ovakav način sinteze obezbeđuje visoku aktivnost čestica, bržu hidrataciju i kratko vreme vezivanja [9]. Cementi na bazi kalcijum-aluminata zbog smanjenog vremena vezivanja i povezane mikrostrukture obezbeđuju veliki potencijal na polju biomaterijala.

Cilj ovog rada bio je da se testom prodora boje ispita marginalna mikropropustljivost novosintetisanih nanostrukturnih biomaterijala na bazi kalcujum-aluminata, kalcijum-silikata i MTA a nakon primene materijala u interradiksne perforacije ekstrahovanih zuba.

MATERIJAL I METODE RADA

U ovom istraživanju testirani su materijali na bazi kalcijum-aluminatnih sistema, beli mineralni trioksidni agregat – MTA (White MTA, Angelus® Soluções odontológicas Londrina, Brazil) i materijali na bazi kalcijum-silikata. Istraživanje je realizovano na 48 ekstrahovanih humanih maksilarnih i mandibularnih molara sa potpuno razvijenim korenovima. Pristupni kaviteti su preparisani visokoturažnom bušilicom, odnosno turbinom, nakon čega je ručnim turpijama (Kfiles, VDW Gmbh, Germany) utvrđena radna dužina kanala korena. Radna dužina je određena do fiziološkog foramena, na 1 mm kraće od anatomskog foramena. Svi kanali korena su preparisani tehnikom Crown-Down. U toku preparacije kanala korena vršena je obilna irigacija sa 0,5% NaOCl-om. Kanali su posušeni papirnim poenima i opturisani monokonom tehnikom uz siler AH PlusTM (Dentsply, Germany), a zatim čuvani u inkubatoru sedam dana, u suvoj sredini na 37°C.

Nakon inkubacije uzoraka, izvršena je perforacija poda komore pulpe između korenova (interradiksna perforacija). Perforacija je urađena kolenjakom i okruglim borerom veličine #4. Širina perforacija je odgovarala promeru borera, dok je dubina zavisila od debljine poda pulpne komore. Zatim su interradiksne perforacije isprane destilovanom vodom i podeljene u tri grupe, nakon čega su zatvorene testiranim materijalima.

Grupa 1: za zatvaranje perforacija je korišćen kalcijum-silikat – po 12 uzoraka

Grupa 2: za zatvaranje perforacija je korišćen MTA – po 12 uzoraka

Grupa 3: za zatvaranje perforacija je korišćen materijal na bazi kalcijum-aluminata – po 24 uzorka

Pre aplikacije testiranih materijala zubi su postavljeni u sunđer natopljen destilovanom i dejonizujućom vodom do nivoa gleđno-cementne granice. Materijal na bazi kalcijum-aluminata je zamešan sa destilovanom vodom u odnosu 3 : 1 i kontrolni materijali su zamešani prema uputstvu proizvođača, zatim su plasirani u furkalne perforacije uz pomoć nabijača. Preko materijala je postavljena vlažna vatica, a zatim su zubi inkubirani na 37°C tokom 24 h. Po vezivanju materijala pristupni kaviteti su definitivno zatvoreni kompozitom. Zubi su čuvani u sunđeru natopljenom destilovanom i dejonizujućom vodom i inkubirani na 37°C, narednih šest meseci.

Posle šest meseci izvršeno je merenje marginalne mikropropustljivosti. Zubi su premazani sa dva sloja laka za nokte, osim u predelu oko same furkacije (1 mm oko furkacije). Nakon toga su svi uzorci potopljeni u 50% rastvor srebro-nitrata (AgNO3) tokom 2 h, a potom isprani u fotografskom razvijaču tokom narednih 6 h. Zatim je izvršeno longitudinalno presecanje zuba kroz samu perforaciju sa dijamantskim diskom debljine 0,7 mm, linearnom preciznom testerom sa vodenim hlađenjem (Isomet testera 4000, Buehler, Lake Bluff, IL, USA). Merenje dubine prodora je analizirano binokularnom lupom (Leica DM 500, Leica Byosistems). Dobijene vrednosti su izražene u milimetrima, a dobijeni rezultati su statistički obrađeni primenjivanjem analize varijanse za ponovljena merenja Sidakovim testom.

REZULTATI

Najmanji prosečan prodor (mm) bio je za MTA (1,40 ± 0,63 mm), a najveći za kalcijum-aluminate (2,10 ± 0,63 mm), dok je za kalcijum-silikate zabeležen prosečan prodor boje od 1,73 ± 0,67 mm.

Testiranjem međugrupnih razlika prodora boje između grupa Sidakovim testom dobijena je statistički značajna razlika između MTA i kalcijum-aluminata ($t = -0,693$; $p = 0,036$). Između MTA i kalcijum-silikata nije zabeležena statistički značajna razlika, kao ni između kalcijum-aluminata i kalcijum-silikata.

DISKUSIJA

Za ispitivanje mikropropuštanja korištene su mnoge metode prodora boje [10], filtracija tečnosti [11], bakterijsko propuštanje [12] i proteinsko propuštanje [13].

U ovom istraživanju za procenu mikropropustljivosti je primenjena metoda linearnog prodora boje. Ova metoda je ujedno i najčešće korišćena metoda za procenu kvaliteta rubnog zatvaranja zbog jednostavnosti primene. Uprkos navedenim prednostima, metoda prodora boje ima i nekoliko nedostataka: molekularna veličina većine čestica boje je veća od veličine bakterija, a prodor boje se uočava u jednoj ravni, odnosno tački [14]. Metod prodora boje je statički, ne odražava dinamičku interakciju sa periradikularnim tkivom, te nije moguće odrediti zapreminu boje između tvrdih zubnih tkiva i dentalnih materijala. U analizi rubnog zatvaranja dentalnih materijala korišćeni su mnogi tipovi boje (metilensko plavo, srebro-nitrat, bazični fuksin, rodamin B...). Metilensko plavo je boja koja se obično koristi zbog svoje pristupačne cene [15]. Rastvor srebro-nitrata je korišćen u ovom istraživanju zbog svoje stabilnosti kod materijala sa visokim pH vrednostima. Čestice srebro-nitrata poseduju veliku molekularnu masu slične veličine kao i same bakterije. Srebro-nitrat nije hidrofilan rastvor, kao što je bazni fuksin [16].

Torabinejad i sardanici su ukazali da bi materijal koji je sposoban da spreči penetraciju malih molekula boje mogao sprečiti i prolazak bakterija i njihovih nusprodukata [17].

Idealan materijala za zatvaranje interradiksnih perforacija trebalo bi da poseduje dimenzionalnu stabilnost, da bude lagan za plasiranje, netoksičan, nekancerogen, da ima rendgensku kontrastnost i da je biokompatibilan [18], odnosno da poseduje sposobnost da izazove osteogenezu i cementogenezu [19].

Torabinejad je 1993. godine predstavio MTA kao biomaterijal koji može da dovede do depozicije cementa kada se koristi u terapiji furkalnih perforacija korena zuba [17]. Ovaj materijal ima superiornije sposobnosti rubnog zatvaranja u poređenju sa drugim restaurativnim materijalima u indikaciji perforacija korena zuba. [20]. Aldayri i saradnici tvrde da MTA prilikom plasiranja u interradiksne perforacije može indukovati formiranje cementnog tkiva [21].

Jacobovitz M. i saradnici su 2009. godine analizirali bakterijsko mikropropuštanje MTA i eksperimentalnog materijala na bazi kalcijum-aluminata (EndoBinder). Oba materijala su se pokazala efikasnima kod mikropropuštanja Enterococcus faecalis in vitro nakon 30 dana. Autori ovaj rezultat pripisuju dodatku disperzantnog sredstva, odnosno aditiva, koji omogućavaju smanjenje udela vode prilikom mešanja materijala, čime se dobija gušća struktura [22].

U ovom istraživanju meren je linearni prodor boje, izražen u milimetrima, odnosno tačka do koje je boja prodrla nakon longitudinalnog presecanja zuba. Nedostatak ove metode je taj što se prodor boje nije mogao odrediti u najdubljoj tački prolaska boje u zubno tkivo. Prečnik eksperimentalnih preparacija odgovarao je prečniku borera (veličina #4), dok je dubina, odnosno dimenzija perforacije zavisila od debljine poda komore pulpe. Camps i Pashley tvrde da se prodor boje oslanja na nasumično presecanje korena na dva dela ne znajući da li presek prolazi kroz najdublji prodor boje [23].

Rezultati sprovedenog istraživanja pokazuju da je najbolje rubno zaptivanje ostvareno kod furkalnih perforacija koje su ispunjene MTA-om. Vrednosti prodora boje kod kalcijum-silikata bile su nešto veće ali bez statistički značajne razlike. Najveći prodor boje zabeležen je kod furkacija koje su ispunjene kalcijum-aluminatnim cementima. Dobijeni rezultati marginalne mikropropustljivosti odgovaraju rezultatima rastvorljivosti i poroznosti testiranih materijala. Razlog ovakvih rezultata može biti i sama struktura cementa izgrađena od brojnih pora i kapilara koji su mogli dovesti do određene propustljivosti. Sarkar i saradnici 2005. godine dobro rubno zaptivanje MTA pripisuju sposobnosti stvaranja hidroksiapatita i njegovoj depoziciji na površini materijala u kontaktu sa tkivom [24]. Jeffrries i saradnici ukazuju na sposobnost formiranja površinskog apatita cemenata na bazi kalcijum-aluminata kod zatvaranja marginalnih mikropukotina u simuliranim fiziološkim uslovima [25].

Prema rezultatima dosadašnjih istraživanja, MTA pokazuje bolje rubno zatvaranje, odnosno manji prodor boje u odnosu na ostale testirane dentalne materijale, što je u skladu sa rezultatima ove studije [26]. Ovo je možda posledica različite konzistencije dentalnih materijala. Nakon mešanja, MTA ima konzistenciju paste, dok je prilikom mešanja kalcijum-aluminata bilo zrnaste konzistencije, što je onemogućilo rubno zatvaranje kompletnim.

Pace i saradnici smatraju da korištenjem biokompatibilnih materijala u reparaciji kod furkalnih perforacija dolazi do smanjenja inflamatornog odgovora okolnih tkiva. Upotrebom MTA za furkalne perforacije kod zuba pasa dolazi do formiranja cementa preko materijala bez infiltrata inflamatornih ćelija. Stoga, navedeni istraživači smatraju da MTA predstavlja idealni materijal u lečenju furkalnih perforacija, jer je netoksičan i nerastvorljiv u vlažnoj sredini [27].

Ispitujući marginalnu mikropropustljivost, odnosno upoređujući sposobnost rubnog zatvaranja MTA, kalcijum-fosfatnog cementa i koštanog cementa u reparaciji furkalnih perforacija u studiji in vitro na 70 humanih ekstrahovanih molara, Chordiya i saradnici su ukazali na najmanju mikropropustljivost MTA. Oni navode da je dobro rubno zaptivanje MTA posledica izvrsne adaptacije materijala na spoljašnje rubove perforacionog otvora zbog ekspanzije materijala [28].

Ispitujući bakterijsko mikropropuštanje materijala na bazi kalcijum-aluminata u kombinaciji sa glas-jonomernim cementima, Pameijer i saradnici u opservacionom periodu od 60 dana na 30 ekstrahovanih premolara pokazuju da materijal na bazi kalcijum-aluminata poseduje dobro rubno zatvaranje i da je bioaktivan, odnosno da poseduje sposobnost stvaranja hidroksiapatita, pružajući mogućnost remineralizacije tkiva. Autori ovakav rezultat pripisuju hemijskoj kompoziciji materijala [29].

Haghgoo i saradnici su analizirali mikropropuštanje kod jatrogenih furkalnih perforacija za MTA i miksturu obogaćenu kalcijumom. Kod oba materijala zabeležen je prodor boje, bez statistički značajnih razlika. Ovaj rezultat autori pripisuju hidrofilnosti oba materijala, odnosno dobroj adaptaciji materijala za zidove perforacije [15].

Jedan od mogućih razloga dobrog rubnog zatvaranja MTA je ekspanzija materijala posle vezivanja, što povećava kvalitet zaptivanja. Druga mogućnost je povezana sa depozicijom kristala kod MTA. Generalno, pregledom literature može se zaključiti da idealan materijal koji bi sprečio prodor boje, odnosno tečnosti, bakterija i mikroorganizama još uvek ne postoji.

ZAKLJUČAK

Najmanja marginalna mikropropustljivost, odnosno najbolje rubno zaptivanje zabeleženo je kod materijala MTA. Mikropropustljivost materijala na bazi kalcijum-aluminata je bila značajno veća u odnosu na kalcijum-silikat i MTA.

REVIEW ARTICLE
PREGLEDNI RAD

https://doi.org/10.2298/SGS2302087T
UDC: 615.33.015.8; 616.31:579.84

Isolation, identification and antibiotic susceptibility of Gram-negative anaerobic bacteria in oral cavity

Zoran Tambur, Ema Aleksić, Jovana Milutinović, Adam Malešević, Dejana Subotić, Vladimir Biočanin

University of Business Academy, Faculty of Dentistry, Pančevo, Novi Sad, Serbia

SUMMARY

Gram-negative bacteria include more than 20 genera. The most commonly isolated genera are: *Bacteriodes* spp., *Porphiromonas* spp., *Fusobacterium* spp. and *Prevotella* spp. The following genera were isolated somewhat less frequently: *Tanerella* spp., *Leptotricha* spp., *Veilonella* spp.,*Wollinela* spp., *Selenomonas* spp. and *Treponema* spp. Anaerobic bacteria have an anaerobic type of metabolism and therefore their incubation is significantly longer and more demanding than aerobic bacteria. The genera *Prevotella* spp., *Porphyromonas* spp. and *Fusobacterium* spp. are part of the resident flora of the oral cavity and in unfavorable conditions cause periodontal diseases, and sometimes dentogenic infections and systemic diseases, such as Alzheimer's disease, cardiovascular diseases, metabolic diseases and inflammatory bowel diseases. The three most important steps for the successful diagnosis of anaerobic bacteria are: proper sampling with avoiding sample contamination, rapid transport of samples to the microbiological laboratory and proper handling of samples. A combination of beta-lactam with the addition of beta-lactamase inhibitors, metronidazole, clindamycin and moxifloxacin is used for treatment for infections caused by anaerobic Gram-negative bacteria. It is important to note that antibiotics should be used only with a clear indication and to choose the right antibiotic in the optimal dose. The aim of this review is to point out the role of Gram-negative anaerobic bacteria in periodontal diseases, and its isolation, identification and antibiotic susceptibility.

Keywords: Gram-negative anaerobic bacteria; systemic diseases; isolation, identification; antibiotics; resistance

INTRODUCTION

The most important infectious diseases of the oral cavity are dental caries, periodontal diseases, dentoalveolar infections and others. The oral cavity is naturally inhabited by microorganisms. It is considered that the oral cavity is sterile at birth and that oral colonization of microorganisms takes place after birth. At the end of 19th century, Gram-negative anaerobic bacteria were discovered and more than 20 genera have been described so far. The following genera are most commonly isolated from oral cavity: *Bacteriodes* spp., *Porphyromonas* spp., *Tanerella* spp., *Fusobacterium* spp. and *Prevotella* spp. In addition, the following genera *Leptotricha* spp., *Wollinela* spp., *Veilonella* spp., *Selenomonas* spp., and *Treponema* spp are detected [1]. Anaerobic bacteria have an anaerobic type of metabolism and therefore their incubation is significantly longer and more demanding than aerobic bacteria. Mutualism and oppurtunism are important characteristics of the symbolic relationship between the host and colonizing species from the genera *Bacteriodes, Prevotella, Porphyromonas* and *Fusobacterium*. *Fusobacterium necroforum* is found as part of the normal flora of the oral cavity and can cause severe infection of the head and neck. Anaerobic bacteria can participate in the development of systemic disease, such as Alzheimer's disease, cardiovascular diseases, metabolic diseases and inflammatory bowel diseases. *Fusobacterium* spp. and *Prevotella* spp. can cause respiratory tract infections [1, 2]. They have a tendency to form abscesses and the most common localization of abscesses is oropharynx, abdominal cavity, lungs and genital tract of women. Several species of the genus *Fusobacterium* have been found to be associated with colon cancer [3]. A combination of beta-lactam with the addition of beta-lactamase inhibitors, metronidazole, clindamycin and moxifloxacin is used for treatment for infections caused by anaerobic Gram-negative bacteria.

Main characteristics of Gram-negative anaerobic bacteria

It has been described 49 species within the genus *Prevotela*. *Prevotela* spp are moderately saccharolytic bacteria (ferment carbohydrates), nonmotile bacilli and strict anaerobes. The main species are: *P. intermedia, P. nigrescens, P. corporis, P. oralis, P. oris, P. dentalis*. Some strains *P. denticola, P. intermedia* and *P. nigrescens* are difficult to differentiate using simple physiological tests. Some species occur in increased numbers in periodontal diseases and are isolated from abscesses [1]. Within the genus *Porphyromonas*, 15 species have been isolated so far. They are mainly composed of asaccharolytic bacteria that use proteins and peptides for their growth. They are nonmotile bacilli and strict anaerobes. The main species are: *P. gingivalis, P. endodontalis, P. catoniae*. They are

Address for correspondence: Zoran TAMBUR, Faculty of Dentistry, Žarka Zrenjanina 179, Pančevo, Serbia;
zoran.tambur@sfp.rs; tambur.zoran@gmail.com

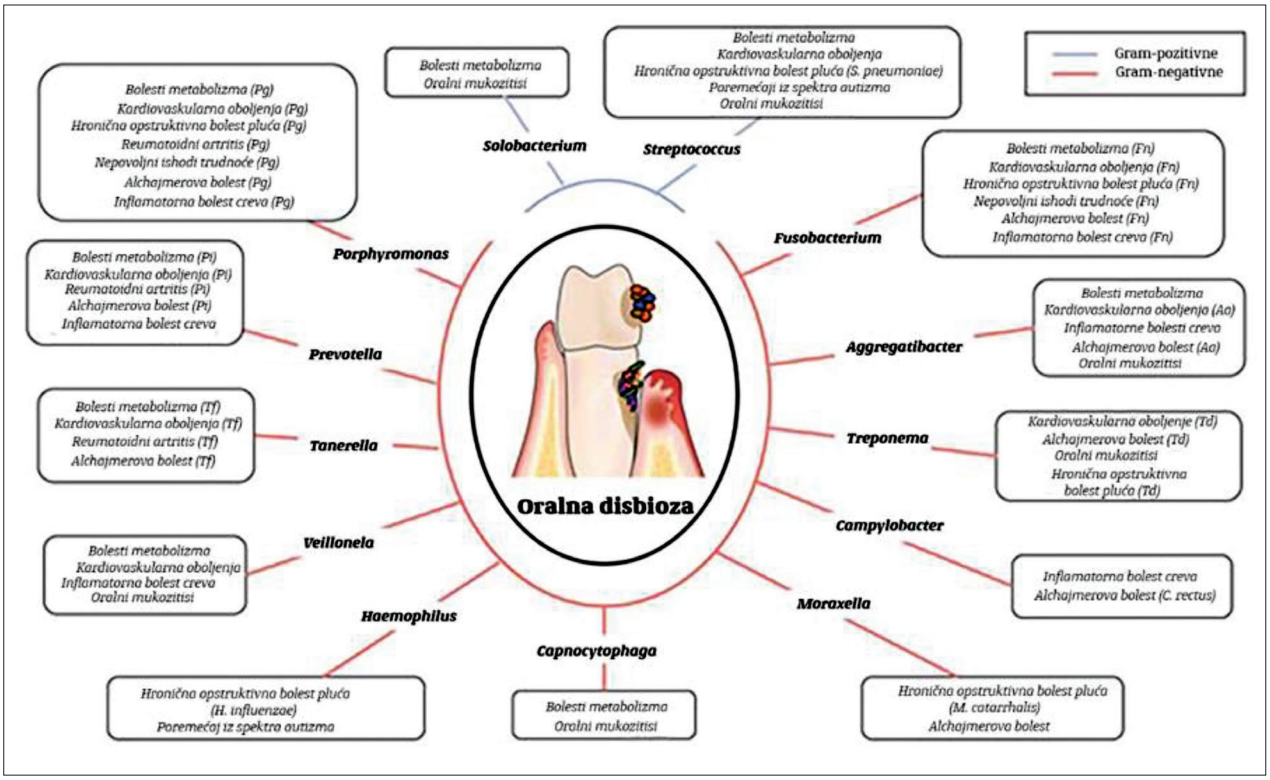

Figure 1. Some major periodontal diseases are associated with other systematic infections [4].
Slika 1. Neke glavne parodontalne bolesti povezane su s drugim sistemskim infekcijama [4].

associated with chronic periodontitis and dentoalveolar abscesses. *P. gingivalis* is mostly isolated from subgingival locations, from advanced periodontal lesions, but also from the tongue and tonsils. *P. endodontalis* is mainly isolated from the root canal of the tooth and a *P. catoniae* is mainly found in healthy localities and in shallow periodontal pockets. The genus *Fusobacterium* currently has 14 species, which 10 of them have been isolated from humans. They represent thin, pleomorphic bacilli and for their cultivation they require enriched media. *F. nucleatum* produces NH_3 and H_2S from cysteine and methionine, so it is considered to cause halitosis. These bacteria have a characteristic shape of long filaments, similar to a Cuban cigarette. The colonial appearance is variable. They are isolated in root canal infections, dentoalveolar abscesses and are present in the spread of odontogenic infections. In order to get the necessary energy, fusobacteria metabolize amino acids (aspartate, glutamate, lysine). If there are no amino acids, peptides are used for this purpose. Within the genus *Treponema,* the main species are: *T. denticola, T. socranskii, T. vincenti, T. maltophilum, T. amylovarum, T. parvum, T. pectinorum, T. putidum, T. medium*. *T. denticola* is asacharolytic. These bacteria are found in the gingival sulcus and are associated with periodontal disease. They are Gram-negative spiral bacilli in three sizes. They are strict anaerobes and are difficult to cultivate *in vitro*. Within the genus *Tannerella*, the main species is *T. forsythia.* They are non-motile, pleomorphic, spindle-shaped Gram-negative bacilli. Sometimes it takes 14 days for visible growth *in vitro.* It grows better with co-cultured with *Fusobacterium nucleatum* and when N-acetylmuramic acid is added to the media. It is found in supragingival and more often in subgingival plaque. The frequency of isolation is closely related to the depth of the periodontal pocket, it is more often isolated from the place where periodontal disease develops, so it is a generally accepted as periodontal pathogen. *Tannerela forsythia, Treponema denticola* and *Porphyromonas gingivalis* are designated as three agents of the "red complex" of bacteria, which is always associated with periodontal disease. The main species in the genus *Wolinella* is *W. succinogenes* are strict anaerobes that move using polar flagella. They can be found in the gingival sulcus as well as in aggressive periodontal disease.

Bacteria from the genus *Veillonella* are strictly anaerobic Gram-negative cocci, they don't break down carbohydrates, but use lactate and thus raise pH value and have an anticariogenic effect. They are isolated from most of the surfaces in oral cavity [2, 3, 4]. Lactic acid is the strongest acid produced by bacteria in oral cavity and breaks down tooth enamel. *Veillonella* spp. can convert lactic acids to weaker acids, mostly propionic carboxylic acid.

Laboratory diagnostics

The three most important steps for successful diagnosis of anaerobic bacteria are: proper sampling with avoiding sample contamination, rapid transport of samples to the microbiological laboratory, proper handling of samples. During the examination, it is the best to take sample from the base of periodontal socket at the right time, taking

care not to cause contamination. Only sterile equipment should be used for sampling. Samples should be inoculated immediately on media for anaerobic bacteria, cultured for different times up to 14 days, under anaerobic conditions. If we transport samples to the laboratory, we must use transport systems. Samples must not be refrigerated. Whenever possible, the sample should be taken before starting antibiotic therapy [5].

Genus *Prevotella*

Gram staining shows pale-stained Gram-negative pleomorphic bacilli or coccobacilli. Microscopically, they cannot be distinguished from *Bacteriodes* spp. They are isolated from periodontal pockets, dental plaque, chronic periodontitis and dentoalveolar abscesses. The procedure for isolating bacteria of the genus *Prevotella* includes the following steps: reparation of sterile medium for growing bacteria, taking a sample from the environment where their presence is expected (oral cavity or gastrointestinal tract), inoculation of the sample onto a sterile supstrate using a sterile loop or pipette, incubation of the supstrate at the optimal temperature and conditions, identification of colonies of bacteria of the genus *Prevotella.* In the genus *Prevotella,* the colonies are similar to *Bacteriodes* spp.*,* except that some species are pigmented (it can be pale brown to black). Cultivation on anaerobic blood agar produces brilliant colonies. *Prevotella* spp. are moderately saccharolytic, while *Porphyromonas* spp. non-saccharoytic. After incubation on blood anaerobic agar for 7-14 days, irregularly shaped colonies with a diameter of about 1mm are formed [6, 7]. Virulence factors are capsular polysaccharide in genus *Prevotella* and inhibit opsonization and therefore phagocytosis, as well as fimbriae and enzymes. They cause skin abscesses and soft tissue infections. They also cause lung abscess, empyema, brain abscess, mastoiditis and inflammation of the middle ear, osteomyelitis of facial bones. The basic approach is abscess drainage and surgical treatment. They are sensitive to metronidazole, clindamicyn, rifampicin and resistant to vancomycin and erythromycin.

Genus *Porphyromonas*

They are associated with chronic periodontitis and dentoalveolar abscesses. *P. endodontalis* is mainly isolated from infected root canals. Isolation of *Porphyromonas* bacteria can be done as follows: a sample can be collected from mouth, teeth, gingiva or other oral surfaces using a sterile spatula or swab. The sample must be transported to the laboratory within 30 minutes after sampling, The sample is inoculated onto *Porphyromonas* media, such as Brucella agar with hemin (10–15%) and vitamin K (0,5%) or tryptone soy agar supplemented with blood (TSBA) with hemin and vitamin K added. Incubation at a temperature of 37 °C for 7-14 days. Colonies are <1.0 mm in diameter after 48 hours of incubation, smooth, shiny and gray. Dark brown or black pigments develop only after 3-7 days [6, 7] and therefore together with bacteria of the genus *Prevotella,* they are classified as black-pigmented anaerobes [1, 5].

Genus *Fusobacterium*

It is isolated from tonsils, normal gingival sulcus and periodontal infections. They require enriched substrates for cultivation. Isolation requires several steps: taking a sample bacteria of the genus *Fusobacterium* can be grown on culture media containing large amounts of blood (blood agar) or similar substances. Confirmation of identification: antibiotic susceptibility and PCR. The appearance of the colonies is variable, but most are 1-3 mm in diameter, with an irregular or jagged margin. They vary from transparent to grainy and opaque. They grow on media with 20% bile and on blood agar for anaerobic bacteria, they cause hemolysis [8]. Virulence factors are lipopolysaccharide endotoxins (LPS) that are endotoxic. *F. necrophorum* causes Lemierre's disease (causes jugular vein thrombosis). The infection can spread to the lungs, brain, liver and bones. In the most severe case, a fatal outcome can occur. It also causes gingivitis and periodontal disease, skin and soft tissue infections, osteomyelitis [9]. Severe invasive diseases, such as Lemierre's disease, are usually treated with a combination of penicillin and metronidazole for 2-6 weeks [10]. It is important to point out that the isolation of these bacteria is an extremely difficult process due to their sensitivity to oxygen.

Diseases caused by Gram-negative anaerobic bacteria

Periodontitis

Periodontitis is a disease that affects general population and leads to tooth loss, most often in people over 40 years old. It is considered to be the most common global disease in the world. The clinical picture is characterized by: inflammation, gingival recession, bleeding, loss of alveolar bone around the teeth, sometimes halitosis. In certain studies, in deep periodontal pockets there is the presence of "red complex" bacteria consisting of *Tannerella forsythia, Porphyromonas gingivalis* and *Treponema denticola* [11, 12].

Necrotic diseases of the periodontium

Necrotic diseases of the periodontium are diseases characterized by the presence of bacteria such as *Fusobacterium, Veillonella,* anaerobic streptococci. They occur in some people with HIV infection [11, 12].

Phlegmon of the mouth floor - Ludwig's angina (LA)

It is characterized by massive solid swelling of the entire floor of the mouth (both sides). Complications including airway obstruction is possible. Bacteria associated with Ludwing's angina are: *Prevotella* spp.*, Porphyromonas* spp.*, Fusobacterium* spp. and anaerobic streptococci. Broad- spectrum antibiotics are used in therapy. Penicilin and metronidazole are combined. It is important to ensure patency of the airways, fluid replacement, taking a sample for microbiological examination and removing the possibility of a source of infection [11, 12].

Periodontal abscess

A periodontal abscess is localized pus collection that is usually located at the apex of the root of the tooth. The cause of an abscess is a bacterial infection that then spreads to the top of the tooth root. Bacteria are not naturally found in periodontium, but they can get there if there is damage to the enamel and dentin, such as caries. The causative agents include bacteria from subgingival plaque, *Porphyromonas, Prevotella, Fusobacterium,* spirochetes, *Capnocytophaga, Actinomyces* spp. The clinical picture is characterized by: sudden onset, swelling, redness, pain is continuous or is related to the bite. The treatment consists in draining the abscess with periodontal procedures. A poor prognosis results in tooth extraction [11, 12].

Antibacterial drugs

Antibiotics are drugs that are toxic for bacteria, but non-toxic or slightly toxic to the human body. The choice of antibiotics is based on the "best guess" principle.

When to include antibiotics in dental practice?

Antibiotics are the most commonly prescribed drugs by dentists, after analgesics. However, they are often inadequately and unnecessarily prescribed. Some of the most commonly prescribed antibiotics are amoxicillin, alone or in combination with clavulanic acid. Antibiotics should be used in case of: the spread of infection in the deep spaces of the head and neck, high body temperature and appearance of general signs of infection, immunocompromised patients (uncontrolled diabetes, patients on long-term corticosteroid and other immunosuppressive therapy, HIV-positive hemodialysis patients), occurrence of canine cavity infection.

In addition to eliminating the cause of infection, antibiotics are also used in prophylaxis. Preventive prescription of antibiotics occurs only in patients with weakened immune system, in whom the occurrence of infection can lead to severe complications. The most commonly prescribed broad-spectrum antibiotics belong to the group of penicillin and cephalosporins. The duration of action of antibiotics is very subjective and depends on the patient. For successful healing, it is necessary to follow the dentist's instructions and complete prescribed therapy. Independent discontinuation of therapy or improper use of antibiotics can lead to relapse [13].

Some of the typical drugs used to treat Gram-negative bacteria are: metronidazole, amoxicilin, cephalosporins, moxifloxacin, clindamycin

Bacterial resistance to antibiotics

Resistance is protection of bacteria to the action of antibiotics, so that the bacteria survive or even multiply. Bacterial resistance can be innate or acquired. In each susceptible population, bacteria mutate and resistant bacteria emerge. Acquired resistance is induced due to long-term use of antibiotics. There is a noticeable trend of increasing resistant strains of Gram-negative anaerobic bacteria [1, 13–17].

There are several methods to slow down the development of resistance: optimal application of antibiotics, synthesis of new antibacterial drugs, synthesis of resistance plasmid propagation inhibitors, use of combinations of antibacterial drugs. It is important to note that antibiotics should be used only with a clear indication and to choose the right antibiotic in the optimal dose. Irrational and uncritical prescribing of antibiotics represents a huge global and national problem. A special problem is multi-resistant bacteria, especially hospital strains bacteria, resistant to at least three classes of antibiotics. Only "reserved antibiotics" still work on them. The body is not given enough time to fight the infection on its own. The choice of appropriate antibiotic is based on the antibiogram, the state of the organism, the site of infection, as well as the side effects of the drug [1, 13–17].

CONCLUSION

Gram-negative anaerobic bacteria are the cause of periodontal and sometimes systemic diseases, and due to their slow growth, suitable media and anaerobic atmosphere, their isolation, identification and antibiotic susceptibility are demanding and time-consuming. In the case of diseases caused by Gram-negative anaerobic bacteria, in the case of need for antibiotic therapy, special attention should be paid to the rational use of appropriate antibiotics due to global trend of increasing bacterial resistance to antibiotics.

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Received: 6.4.2023 • Accepted: 6.6.2023

Izolacija, identifikacija i ispitivanje osetljivosti na antibiotike Gram-negativnih anaerobnih bakterija usne duplje

Zoran Tambur, Ema Aleksić, Jovana Milutinović, Adam Malešević, Dejana Subotić, Vladimir Biočanin

Univerzitet Privredna akademija u Novom Sadu, Stomatološki fakultet Pančevo, Pančevo, Srbija

KRATAK SADRŽAJ

U Gram-negativne bakterije spada više od 20 rodova. Najčešće su izolovani sledeći rodovi: *Bacteriodes* spp., *Porphyromonas* spp., *Fusobacterium* spp. i *Prevotella* spp. Nešto ređe se izoluju sledeći rodovi: *Tanerella* spp., *Leptotricha* spp., *Veilonella* spp., *Wollinela* spp., *Selenomonas* spp. i *Treponema* spp. Anaerobne bakterije imaju anaerobni tip metabolizma i zbog toga im je inkubacija znatno duža i zahtevnija u odnosu na aerobne bakterije. Rodovi *Prevotella* spp., *Porphyromonas* spp. i *Fusobacterium* spp. deo su rezidentne flore usne duplje, a u nepovoljnim uslovima izazivaju oboljenja parodoncijuma, a ponekad dentogene infekcije i sistemska oboljenja, kao što su Alchajmerova bolest, kardiovaskularna oboljenja, bolesti metabolizma i inflamatorne bolesti creva. Tri najvažnija koraka za uspešnu dijagnostiku anaerobnih bakterija su: pravilno uzimanje uzoraka, uz izbegavanje kontaminacije uzorka, brzi transport uzoraka u mikrobiološku laboratoriju i pravilno rukovanje uzorcima. Za lečenje infekcija uzrokovanih anaerobnim Gram-negativnim bakterijama upotrebljava se kombinacija beta-laktamskih antibiotika uz dodatak inhibitora beta-laktamaza, metronidazol, klindamicin i moksifloksacin. Važno je napomenuti da antibiotike treba koristiti samo uz jasnu indikaciju i da treba odabrati pravi antibiotik u optimalnoj dozi.

Cilj ovog rada je da se ukaže na ulogu Gram-negativnih anaerobnih bakterija u oboljenjima parodoncijuma, njihovu izolaciju, identifikaciju i osetljivost na antibiotike.

Ključne reči: Gram-negativne anaerobne bakterije; sistemska oboljenja; izolacija, identifikacija; antibiotici; rezistencija

UVOD

Najvažnija infektivna oboljenja usne duplje su zubni karijes, oboljenja parodoncijuma, dentoalveolarne i druge infekcije. Usna duplja je prirodno naseljena mikroorganizmima. Smatra se da je po rođenju sterilna i da se oralna kolonizacija mikroorganizama odvija nakon rođenja. Krajem 19. veka otkrivene su Gram-negativne anaerobne bakterije i do sada je opisano više od 20 rodova. Najčešće se iz usne duplje izoloju sledeći rodovi: *Bacteriodes* spp., *Porphyromonas* spp., *Tanerella* spp., *Fusobacterium* spp. i *Prevotella* spp. Opisano je prisustvo i sledećih rodova: *Leptotricha* spp., *Wollinela* spp., *Veilonella* spp., *Selenomonas* spp., *Treponema* spp. Anaerobne bakterije imaju anaerobni tip metabolizma, te im je inkubacija zahtevnija u odnosu na aerobne bakterije. Mutualizam i oportunizam su važne karakteristike simbiotskog odnosa domaćina i kolonizujućih vrsta iz rodova *Bacteriodes*, *Prevotella*, *Porphyromonas* i *Fusobacterium*. *Fusobacterium necroforum* se nalazi kao deo normalne flore usne duplje, a može uzrokovati tešku infekciju glave i vrata. Anaerobne bakterije mogu učestvovati u nastanku sistemskih oboljenja, kao što su Alchajmerova bolest, kardiovaskularna oboljenja, bolesti metabolizma i inflamatorne bolesti creva. *Fusobacterium* spp. i *Prevotella* spp. mogu uzrokovati infekcije disajnih puteva [1, 2]. Imaju sklonost za formiranje apscesa, a najčešća lokalizacija apscesa su orofarinks, trbušna duplja, pluća i genitalni trakt žena. Otkriveno je da je nekoliko vrsta iz roda *Fusobacterium* povezano i sa karcinomom debelog creva [3]. Za lečenje infekcija uzrokovanih anaerobnim Gram-negativnim bakterijama koristi se kombinacija beta-laktamskih antibiotika i inhibitora beta-laktamaza, metronidazol, klindamicin i moksifloksacin.

Glavne karakteristike Gram-negativnih anaerobnih bakterija

Do sada je opisano 49 vrsta u okviru roda *Prevotela*. *Prevotela* spp. su umereno saharolitične bakterije (fermentuju ugljene hidrate), nepokretni bacili i striktni anaerobi. Glavne vrste su: *P. intermedia*, *P. nigrescens*, *P. corporis*, *P. oralis*, *P. oris*, *P. dentalis*. Neki sojevi *P. denticola*, *P. intermedia* i *P. nigrescens* teško se diferenciraju ako se koriste jednostavni fiziološki testovi. Neke vrste se javljaju u povećanom broju u parodontalnim bolestima, a izoluju se iz apscesa [1]. U sklopu roda *Porphyromonas* do sada je izolovano 15 vrsta. Uglavnom ih čine asaharolitične bakterije koje za svoj rast koriste proteine i peptide. Nepokretni su bacili i striktni anaerobi. Glavne vrste su: *P. gingivalis*, *P. endodontalis*, *P. catoniae*. Povezuju se sa hroničnom parodontopatijom i dentoalveolarnim apscesima. *P. gingivalis* se uglavnom izoluje iz subgingivalnih lokaliteta, iz uznapredovalih parodontalnih lezija, ali i sa jezika i tonzila. *P. endodontalis* uglavnom se izoluje iz kanala korena zuba, a *P. catoniae* se uglavnom nalazi u zdravim lokalitetima i u plitkim parodontalnim džepovima. Rod *Fusobacterium* trenutno broji 14 vrsta, od kojih je 10 izolovano kod ljudi. Predstavljaju tanke pleomorfne bacile za čiju kultivaciju zahtevaju obogaćene podloge. *F. nucleatum* produkuje NH_3 i H_2S iz cisteina i metionina, pa se smatra uzročnikom halitoze. Ove bakterije su karakterističnog oblika dugih filamenata, nalik kubanskoj cigareti. Kolonijalni izgled je promenljiv. Izolovani su kod infekcija kanala korena, dentoalveolarnih apscesa i zastupljeni su kod širenja odontogenih infekcija. Da bi došle do potrebne energije, fuzobakterije metabolišu amino-kiseline (aspartat, glutamat, lizin). Ako nema amino-kiselina u tu svrhu koriste peptide. U okviru roda *Treponema* glavne vrste su: *T. denticola*, *T. socranskii*, *T. vincenti*, *T. maltophilum*, *T. amylovarum*, *T. parvum*, *T. pectinorum*, *T. putidum*, *T. medium*. *T. denticola* je asaharolitična. Ove bakterije se nalaze u gingivalnom sulkusu i povezane su sa parodontalnim bolestima. To su Gram-negativni spiralni bacili u tri veličine. Striktni su anaerobi i teško se kultivišu *in vitro*. U sklopu roda *Tannerella* glavna vrsta je *T. forsythia.* To su nepokretni, pleomorfni, vretenasti Gram-negativni bacili. Za vidljiv porast *in vitro* potrebno je nekada i 14 dana. Bolje raste kada se kultiviše zajedno sa *Fusobacterium nucleatum* i kada se podlogama doda *N-acetilmuraminska kiselina.* Nalazi se u

supragingivalnom, a češće u subgingivalnom plaku. Učestalost izolacije je u uskoj vezi sa dubinom parodontalnog džepa, češće se izoluje sa mesta gde se razvija oboljenje parodoncijuma, pa je opšteprihvaćen parodontalni patogen. *Tannerela forsythia*, *Treponema denticola* i *Porphyromonas gingivalis* označavaju se kao tri agensa „crvenog kompleksa" bakterija, koji je uvek udružen sa parodontalnom bolešću. Glavna vrsta u rodu *Wolinella* je *W. succinogenes.* To su striktni anaerobi koji se kreću pomoću polarnih flagela. Mogu se pronaći u gingivalnom sulkusu, kao i kod agresivne parodontalne bolesti. Bakterije iz roda *Veillonella* su striktno anaerobni Gram-negativni koki, ne razgrađuju ugljene hidrate, već koriste laktat i na taj način podižu pH vrednost i imaju antikariogeni efekat. Izoluju se sa većine površina u usnoj duplji [2, 3, 4]. Mlečna kiselina je najjača kiselina koju produkuju bakterije usne duplje i razgrađuje zubnu gleđ. Veilonele mogu da je konvertuju u slabije kiseline, najvećim delom u propionsku karboksilnu kiselinu.

Laboratorijska dijagnostika, glavna mesta izolacije iz usne duplje

Tri najvažnija koraka za uspešnu dijagnostiku anaerobnih bakterija su: pravilno uzimanje uzoraka, uz izbegavanje kontaminacije uzorka, brzi transport uzoraka u mikrobiološku laboratoriju, pravilno rukovanje uzorcima. Prilikom ispitivanja, uzorak je najbolje uzeti sa baze parodontalnog džepa u pravom trenutku, vodeći računa da ne dođe do kontaminacije. Za uzimanje uzorka treba koristiti samo sterilnu opremu. Uzorke treba odmah zasejati na podloge za anaerobne bakterije, kultivisati tokom različitih vremenskih perioda, ponekad i do 14 dana, u anaerobnim uslovima. Ako uzorke transportujemo u laboratoriju, obavezno treba koristiti transportne sisteme. Uzorci se nikako ne smeju stavljati u frižider. Kada god je moguće, uzorak treba uzeti pre početka antibiotske terapije [5].

Rod *Prevotella*

Bojenjem po Gramu vide se bledo obojeni Gram-negativni pleomorfni bacili ili kokobacili. Mikroskopski se ne mogu razlikovati od *Bacteriodes* spp. Izoluju se iz parodontalnih džepova, zubnog plaka, kod hronične parodontopatije i dentoalveolarnih apscesa. Postupak izolacije bakterija roda *Prevotella* uključuje sledeće korake: priprema sterilne podloge za uzgajanje bakterija, uzimanje uzorka iz okoline gde se očekuje njihova prisutnost (usna šupljina ili gastrointestinalni trakt), inokulacija uzorka na sterilnu podlogu pomoću sterilne petlje ili pipete,

inkubacija podloge na optimalnoj temperaturi i pod optimalnim uslovima, identifikacija kolonija bakterija roda *Prevotella,* ispitivanje osetljivosti na antibiotike. Kada je reč o rodu *Prevotella*, kolonije su slične kao kod *Bacteriodes* spp, osim što su neke vrste pigmentovane (mogu biti bledosmeđe do crne). Kultivacijom na anaerobnom krvnom agaru stvaraju sjajne kolonije. *Prevotella* spp. su umereno saharolitične, dok je *Porphyromonas* spp. nesaharolitičan. Nakon inkubacije na krvnom anaerobnom agaru u trajanju od 7 do 14 dana stvaraju se kolonije nepravilnog oblika, promera oko 1 mm [6, 7].

Faktori virulencije su kapsularni polisaharid kod prevotela i inhibiraju opsonizaciju, a samim tim i fagocitozu, te fimbrije i enzimi. Stvaraju kožne apscese i infekcije mekih tkiva. Uzrokuju plućni apsces, empijem, moždani apsces, mastoiditis i upalu srednjeg uha, osteomijelitis kostiju lica. Osnovni pristup je drenaža apscesa i hirurška obrada. Osetljivi su na metronidazol, klindamicin, rifampicin, a otporni na vankomicin i eritromicin.

Rod *Porphyromonas*

Povezuje se sa hroničnom parodontopatijom i dentoalveolarnim apscesima. *P. endodontalis* se uglavnom izoluje iz inficiranih kanala korena zuba. Izolacija bakterija roda *Porphyromonas* može se izvesti na sledeći način: uzorak se može prikupiti iz usta, zuba, gingive ili drugih oralnih površina pomoću sterilne špatule ili brisa i mora se transportovati u laboratoriju u roku od 30 minuta nakon uzorkovanja.

Uzorak se zasejava na podloge za *Porphyromonas*, kao što su Brucella agar sa heminom (5–10%) i vitaminom K (0,5%) ili tripton soja agar sa dodatkom krvi (TSBA), sa dodatkom hemina i vitamina K. Inkubacija je na temperaturi od 37°C tokom 7–14 dana. Kolonije su < 1,0 mm u prečniku nakon 48 sati inkubacije, glatke, sjajne i sive. Tamnosmeđi ili crni pigmenti se razvijaju tek nakon 3–7 dana [6, 7] i zato se zajedno sa bakterijama roda *Prevotella* ubrajaju u crno pigmentovane anaerobe [1, 5].

Rod *Fusobacterium*

Izoluje se iz tonzila, normalnog gingivalnog sulkusa i parodontalnih infekcija. Za kultivaciju zahteva obogaćene podloge. Izolacija zahteva uzimanje uzoraka bakterije roda *Fusobacterium*, koji mogu da se uzgajaju na hranljivim podlogama koje sadrže velike količine krvi (krvni agar) ili slične supstance. Potvrda identifikacije je test osetljivosti na antibiotike i PCR. Izgled kolonija je promenljiv, ali većina je prečnika 1–3 mm, sa nepravilnom ili nazubljenom ivicom. Variraju od providnih do zrnastih i neprozirnih. Rastu na podlogama sa 20% žuči, a na krvnom agaru za anaerobne bakterije stvaraju hemolizu [8]. Faktor virulencije je lipopolisaharidni endotoksin, koji deluje kao endotoksin. *F. necrophorum* uzrokuje Lemiereovu bolest (uzrokuje trombozu jugularne vene). Infekcija se može proširiti na pluća, mozak, jetru i kosti. U najtežem slučaju može doći do letalnog ishoda. Uzrokuje takođe i gingivitis i parodontalnu bolest, infekcije kože i mekih tkiva, osteomijelitis [9]. Teške invazivne bolesti, kao što je Lemierova bolest, obično se leče kombinacijom penicilina i metronidazola tokom 2–6 nedelja [10]. Važno je istaći da je izolacija ovih bakterija izuzetno težak proces zbog osetljivosti na kiseonik.

Oboljenja koja uzrokuju Gram-negativne anaerobne bakterije

Parodontitisi

Parodontitis predstavlja oboljenje koje zahvata opštu populaciju i dovodi do gubitka zuba najčešće kod osoba starijih od 40 godina. Smatra se da je najčešće oboljenje na svetu. Kliničku sliku karakterišu inflamacija, recesija gingive, krvarenje, gubitak alveolarne kosti oko zuba, ponekad halitoza. Kod određenih studija u dubokim parodontalnim džepovima identifikovano je prisustvo bakterija „crvenog kompleksa", koji čine *Tannerella forsythia*, *Porphyromonas gingivalis* i *Treponema denticola* [11, 12].

Nekrozna oboljenja parodoncijuma

Nekrozna oboljenja parodoncijuma su oboljenja koja odlikuje prisutsvo bakterija kao što su *Fusobacterium*, *Veillonella*, anaerobni streptokoki. Javljaju se kod nekih osoba sa HIV infekcijom [11, 12].

Flegmona poda usta – Ludvigova angina

Karakteriše se masivnim čvrstim otokom kompletnog poda usta (obostrano). Moguće su komplikacije koje uključuju opstrukciju disajnih puteva. Bakterije koje se povezuju sa Ludvigovom anginom su: *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp. i anaerobni streptokoki. U terapiji se koriste antibiotici širokog spektra dejstva. Kombinuju se penicilin i metronidazol. Važno je obezbediti prohodnost disajnih puteva, nadoknadu tečnosti, uzimanje uzorka za mikrobiološki pregled i ukloniti mogući izvor infekcije [11, 12].

Periodontalni apsces

Periodontalni apsces je lokalizovana gnojna kolekcija koja se uglavnom nalazi na samom vrhu korena zuba. Uzrok apscesa je bakterijska infekcija koja se potom širi na vrh korena zuba. Bakterije se prirodno ne nalaze u periodoncijumu, ali tamo mogu dospeti ako na gleđi i dentinu postoje oštećenja, kao što je karijes. Uzročnicima pripadaju bakterije iz subgingivalnog plaka, *Porphyromonas*, *Prevotella*, *Fusobacterium*, spirohete, *Capnocytophaga*, *Actinomyces* spp. Kliničku sliku karakterišu nagli početak, otok, crvenilo, bol je kontinuiran ili je u vezi sa zagrižajem. Lečenje se sastoji u dreniranju apscesa parodontološkim postupcima. Loša prognoza rezultuje vađenju zuba [11, 12].

Antibakterijski lekovi

Antibiotici su lekovi koji su toksični za bakterije, a netoksični ili slabo toksični za čovekov organizam. Izbor antibiotika zasniva se na principu „najbolje pretpostavke" (*best guess*).

Kada uključiti antibiotike u stomatološku praksu?

Antibiotici su, od strane stomatologa, najčešće propisivani lekovi, posle analgetika. Međutim, često se neadekvatno i nepotrebno propisuju. Neki od najčešće propisivanih antibiotika su amoksicilin, samostalno, ili u kombinaciji sa klavulanskom kiselinom. Antibiotici bi trebalo da se koriste u slučaju širenja infekcije u duboke prostore glave i vrata, visoke telesne temperature i pojave opštih znakova infekcije, kod imunokompromitovanih pacijenata (nekontrolisani dijabetes, pacijenti na dugotrajnoj kortikosteroidnoj i drugoj imunosupresivnoj terapiji, HIV pozitivni pacijenti na hemodijalizi), pojave infekcije očnjačke jame. Osim eliminacije uzročnika infekcije, antibiotici se koriste i u profilaksi. Preventivno propisivanje antibiotika događa se samo kod pacijenata sa oslabljenim imunološkim sistemom, kod kojih pojava infekcije može dovesti do teških komplikacija. Najčešće propisivani antibiotici širokog spektra delovanja spadaju u grupu penicilina i cefalosporina. Vreme delovanja antibiotika je veoma subjektivno i zavisi od pacijenta do pacijenta. Za uspešno izlečenje potrebno je slediti uputstvo stomatologa i dovršiti propisanu terapiju. Samostalno prekidanje terapije ili nepravilna upotreba antibiotika mogu dovesti do recidiva [13].

Antimikrobni lekovi koji se najčešće koriste u stomatologiji

Najčešće propisivani antibiotici širokog spektra spadaju u grupu penicilina i cefalosporina.

Rezistencija bakterija na antibiotike

Rezistencija je otpornost bakterija na dejstvo nekog antibiotika, tako da bakterije opstaju ili se čak umnožavaju. Bakterijska rezistencija može biti urođena i stečena. U svakoj osetljivoj populaciji bakterije mutiraju i nastaju rezistentne bakterije. Stečena rezistencija je indukovana usled dugotrajne primene antibiotika. Primetan je trend porasta rezistentnih sojeva Gram-negativnih anaerobnih bakterija [1, 13–17]. Postoji nekoliko metoda za uspostavanje rezistencije: optimalna primena antibiotika, sinteza novih antibakterijskih lekova, sinteza inhibitora razmnožavanja plazmida rezistencije, upotreba kombinacija antibakterijskih lekova. Važno je napomenuti da antibiotike treba koristiti samo uz jasnu indikaciju i odabrati pravi antibiotik u optimalnoj dozi. Neracionalno i nekritičko propisivanje antibiotika predstavlja ogroman globalni i nacionalni problem. Poseban problem predstavljaju multirezistentne bakterije, posebno bolnički sojevi bakterija, rezistentne na najmanje tri klase antibiotika. Na njih jedino još deluju „rezervni antibiotici". Ne ostavlja se dovoljno vremena organizmu da se sam izbori sa infekcijom. Izbor odgovarajućeg antibiotika vrši se na osnovu antibiograma, stanja organizma, mesta infekcije, kao i neželjenih dejstava leka [1, 13–17].

ZAKLJUČAK

Gram-negativne anaerobne bakterije su izazivači parodontalnih, a nekada i sistemskih oboljenja, a zbog sporog rasta, odgovarajućih podloga i anaerobne atmosfere njihova izolacija, identifikacija i ispitivanje osetljivosti na antibiotike zahtveni su i dugotrajni. U slučaju oboljenja izazvanih Gram-negativnim anaerobnim bakterijama, u slučaju potrebe za antibiotskom terapijom, treba obratiti posebnu pažnju na racionalnu upotrebu odgovarajućih antibiotika zbog globalnog trenda porasta rezistencije bakterija na antibiotike.

REVIEW ARTICLE
PREGLEDNI RAD

https://doi.org/10.2298/SGS2302095G
UDC: 614.2:336(497.11)"2004/2020"

Overview of changes in the health sector and its financing in the Republic of Serbia in the period 2004–2020

Milena Gajić-Stevanović, Ivan Stevanović

NGO Health Monitor, Belgrade, Serbia

SUMMARY

Introduction In the period from 2004 to 2020, many reforms were implemented in the health sector of the Republic of Serbia and it's financing. The aim of this work was to provide an insight into foundations on which healthcare was based in the Republic of Serbia in the period from 2004 to 2020 and review the reform changes in the healthcare sector and it's financing.

Material and method A retrospective analysis of data from the National Health Account of the Institute of Public Health of Serbia, the regulations of the Republic Health Insurance Fund, reports of the Ministry of Health as well as laws and regulations related to the health sector was performed.

Results The results of the analysis showed that the State health sector represented the foundation of the health system in the Republic of Serbia in the observed period. Of the total health financing, it was estimated that about sixty percent were public financing schemes, and about forty percent belonged to private schemes, with a large part of private schemes being out-of-pocket payments.

Conclusion The general conclusion of the analysis is that in the observed period, within the framework of the reform changes, Serbia had a good system of exemption from participation, but that out-of-pocket payments for certain health services and corrupt payments represented a barrier to health care.

Keywords: Health sector reform; health care financing; basic benefit package

INTRODUCTION

The health care system of the Republic of Serbia is based on the principles of mandatory health insurance. The Republic Health Insurance Fund (RHIF) is a national organization through which citizens exercise their right to health insurance and finance health care. RHIF finances the functioning of health care at all levels, contracts the provision of services with public and private health institutions, controls the implementation of the obligations assumed when contracting with them, defines the basic package of health services.

Due to the lack of an adequate network of private health insurance, private health financing is mainly based on out-of-pocket payments, supplemented by the participation of a small number of large companies. The mentioned companies have (and finance) their own health institutions that specialize in the treatment of occupational diseases, and also provide all services in the domain of primary health care.

The aim of this work was to provide an insight into the foundations on which healthcare was based in the Republic of Serbia in the period from 2004 to 2020 and review the reform changes in the healthcare sector and it's financing.

MATERIAL AND METHOD

A retrospective analysis of data from the National Health Account of the Institute of Public Health of Serbia, the regulations of the Republic Health Insurance Fund, reports of the Ministry of Health as well as laws and regulations related to the health sector was performed.

RESULTS

In the period from 2004 to 2020, of the total health financing, it was estimated that about sixty percent were public financing schemes, and that about forty percent belonged to private schemes, with a large part of private schemes consisting of out-of-pocket payments [1]. About 1.5% of the 60% of public funding schemes were directed at funding private healthcare providers, and the rest at public providers. Of the 40% of private funding schemes, it was estimated that 35% were directed towards private healthcare providers and 5% towards public providers.

Within the public sector of health care financiers in the Republic of Serbia, the predominant financier was the Republic Health Insurance Fund (RHIF) with a share of around 94% [1]. RHIF was financed from the contributions of employees and employers in the amount of 67%, 22% from the PIO Fund, 4% from the contributions of the self-employed, and 7% from state transfers on behalf of special vulnerable groups of the population [1].

Public health care was provided directly through the network of health institutions defined by the Regulation on the Plan of the Network of Health Institutions [2]. According to the network plan in 2020, there were 313 health institutions (without institutions from Kosovo and Metohija) at the primary, secondary and tertiary levels.

Address for correspondence: Milena GAJIĆ-STEVANOVIĆ, Cvijićeva 72, Belgrade, Serbia; *mima_gajic@yahoo.com*

In the observed period, the Ministry of Health owns the majority of health institutions and directly finances them mainly through capital investments. The Ministry of Finance transfers funds to the RHIF for providing health care for vulnerable uninsured groups: the long-term unemployed, uninsured elderly and very young people, as well as migrants, refugees and internally displaced persons.

Donations from foreign countries and international organizations are also transferred to RHIF. Local government contributes to health financing, mainly for investment purposes.

Payment model for primary and secondary health care

The health reform between 2004 and 2020 transformed the health service and focused on primary health care and prevention over curative care, in order to reduce the rate of curable diseases and reduce health expenditure.

Capitation was chosen as the payment model for primary health care (application of the capitation formula began in November 2012 in primary health care institutions) and the model of diagnostically related groups (DSG) for payments in secondary health care.

There were key changes in the capitation formula in 2020 established by the Regulation on the corrective coefficient in the capitation formula [3].

In addition to corrective factors such as the age of the patient, a corrective factor for population density, specific quality indicators for each specialty, the aforementioned Regulation also introduced corrective factors of efficiency and diagnostic-therapeutic procedure (DTP) as corrective factors for all specialties.

For diagnostically related groups, changes are taking place in the way of hospital reporting and budget modeling. It is gradually moving from the retrospective method of paying for health services, which was based on the historical budget, to a prospective payment system based on diagnostically related groups.

Laws regulating health care

The provision of health care in the Republic of Serbia is regulated by: the Law on Health Care, the Law on Health Insurance and the Law on Public Health. The Constitution of the Republic of Serbia defines the Republic of Serbia as the state of the Serbian people and all its citizens, establishes the rule of law and social justice, the principles of civil democracy, human and minority rights and freedoms, as well as belonging to European principles and values [4]. According to the Article 68 of the Constitution, everyone has the right to the protection of their physical and mental health according to the principle of solidarity in financing and the principle of equality in access to health care.

Compulsory health insurance is organized on the principle of: obligation, solidarity and reciprocity, protection of the rights of the insured and protection of public interest, continuous improvement of the quality of compulsory health insurance and economy as well as efficiency of compulsory health insurance [5]. The principle of compulsory insurance refers to the obligation to pay contributions for compulsory health insurance based on 12.3% until 2014 and from then until today 10.3% deduction from salary [6]. Regardless of the amount of available funds and fluctuations in health care costs, in the observed period, the Law on Health Care and the Law on Health Insurance define the basic package of health services. Under these laws, compulsory health insurance covers (A) non-work-related illnesses and injuries and (B) work-related injuries and illnesses [3].

In addition, mandatory health insurance funds cover the costs of rehabilitation, procurement of medical-technical equipment, prescription drugs, sick leave (longer than a month), transportation costs related to the use of health care, etc. Sick leave up to 30 days are covered by the employer and there is no regularly collected data on them by the state. Social care for health is achieved by providing health care to groups of the population with an increased risk of disease, health care of persons in connection with the prevention, control, early detection and treatment of diseases and conditions of greater public health importance, as well as health care of the socially vulnerable population, under equal rights on the territory of the Republic of Serbia [7].

The basic package of health services represents the services that are provided from the funds of mandatory health insurance, according to the Article 52 of the law on health insurance, the nomenclature (name and description) of health services and the price list of health services from mandatory health insurance. The basic package consisted of:

1. measures for prevention and early detection of diseases;
2. examinations and treatment related to family planning, during pregnancy, childbirth and up to 12 months after childbirth;
3. examinations and treatment in case of illness and injury;
4. examinations and treatment of diseases of the mouth and teeth;
5. medical rehabilitation in case of illness and injury;
6. medicines;
7. medical means [5].

According to the Law on Health Insurance, insured persons are fully covered without any co-payment for: preventive measures and early detection of diseases, screening and treatment for family planning, pregnancy, childbirth and postpartum care, including termination of pregnancy for medical reasons, examinations, treatment and medical rehabilitation in case of illness and injury of children, pupils and students until the end of the prescribed schooling, and at the latest until the age of 26, i.e. elderly persons with severe physical or mental disorders, examinations and treatment of mouth and dental diseases related to pregnancy and 12 months after childbirth, examinations and treatment of infectious diseases that are legally required to implement measures to prevent

their spread, examinations and treatment of malignancy, diabetes, psychosis, epilepsy, multiple sclerosis, progressive neuromuscular diseases, cerebral palsy, paraplegia, tetraplegia, permanent chronic renal insufficiency that indicates dialysis or kidney transplantation, systemic autoimmune diseases, rheumatic diseases and their complications, rare diseases, palliative care, examinations and treatment in connection with the collection, application and exchange of organs, cells and tissues for transplantation, examinations, treatment and rehabilitation from occupational diseases and injuries at work, provision of emergency medical and dental assistance, as well as emergency medical transport [5].

If the content and scope of the right to health care from the mandatory health insurance cannot be realized due to insufficient funds, the Government passes an act that determines the priorities in the provision and implementation of health care. Also, the Government every year, on the proposal of the Minister of Health, passes an act that determines the priorities in the treatment of patients with certain types of rare diseases, for which funds are provided in the budget of the Republic of Serbia.

The percentage of co-payment coverage varies depending on the type of health service:

- In the amount of at least 95% of the price of health services from the mandatory health insurance funds for: intensive care in a stationary health facility, surgical procedures performed in the operating room, including the installation of implants for the most complex and expensive health services, the most complex laboratory, X-ray and other diagnostic and therapeutic procedures (magnetic resonance imaging, scanner, nuclear medicine, etc.);
- In the amount of at least 80% of the price of health services from the funds of the mandatory health insurance for: examinations and treatment at the selected doctor and specialist, laboratory, X-ray and other diagnostic and therapeutic procedures, home treatment, dental examinations and treatment related to injuries to teeth and bones faces, as well as dental examinations and dental treatments before heart surgery and transplantation of organs, cells and tissues.
- Also, for the treatment of caries complications in children, students and students until the end of the prescribed period of schooling, and at the latest until the age of 26, tooth extraction as a result of caries, as well as the development of mobile orthodontic appliances, inpatient treatment and rehabilitation in an inpatient health facility, examinations and treatment in a day hospital, including surgery outside the operating room, outpatient medical rehabilitation, some medical devices.
- In the amount of at least 65% of the price of health services from the mandatory health insurance funds for: fabrication of acrylate total and subtotal dentures for persons over 65 years of age, eye and hearing aids for adults, gender change for medical reasons, non-emergency medical transport, treatment of diseases, early detection of which is subject to targeted preventive screening, i.e. screening, in accordance with the relevant national programs, if the insured person did not respond to a single call within one call cycle, or justified his absence, and was diagnosed with a disease by the next call cycle.

Health services that are not described in the Basic package of services are services that the insured person pays from his own funds, at prices determined by the health care provider.

The negative list of health services is a list of interventions, services and products that are generally paid out of pocket in public or private health institutions, and are not included in the list of the basic package of services.

The new Health Insurance Law that came into force in 2019, provided more rights to use health services such as medical gender reassignment and artificial insemination for women [5], compared to the 2005 law.

Costs of health care users for medicines and health aids

In its contracts with public institutions, the Republican Health Insurance Fund approximates in advance the participation that each institution realizes. The approximate amount is deducted from the funds transferred to the institutions. The amount of the approximate participation is 1-3% of the total income of health institutions.

The State of Serbia adopts by-laws and regulations that regulate the scope of the right to health care from the mandatory health insurance, which also includes the right to medicines. The population's costs for medicines and auxiliary means make up about sixty percent of their total direct costs for health care, although RHIF also invests significant financial resources [1].

According to the RHIF rulebook [8] in the list of drugs that are prescribed and issued on the basis of mandatory health insurance, all drugs are divided into 5 basic groups.

1) A. Prescription drugs (List A, drugs that are issued with a fixed co-payment - 50 dinars per prescription);
2) A1. Medicines that are prescribed and issued in the form of a doctor's prescription, which have a therapeutic parallel (therapeutic alternative) to drugs from List A (List A1 drugs are issued with a 10 to 90% co-payment per package);
3) B. Medicines used during outpatient or hospital treatment in health institutions (List B, medicines dispensed without co-payment);
4) C. Medicines with a special regime (List C, medicines that are issued without co-payment);
5) drugs that do not have a license to be placed on the market in the Republic of Serbia, but are necessary for diagnosis and therapy - unregistered drugs, and exceptionally drugs are registered in the Republic of Serbia with the same generic name (IGN) as the drug that is placed on the list of drugs, and which is not available on the market of the Republic of Serbia in quantities necessary to provide health care to insured persons, i.e. which has been withdrawn from the market (List D, drugs issued without co-payment).

The entire process of placing a medicine or deleting it from the list of medicines must be in accordance with the Rulebook on the conditions, criteria, method and procedure for placing a medicine on the List of Medicines, amendments and additions to the List of Medicines, that is, for deleting a medicine from the List of Medicines [9].

The key information required by the current regulations is evidence of safety and efficacy, together with a cost-based pharmaco-economic analysis according to the defined daily dose and a budget impact analysis. However, a cost-effectiveness analysis is required, which is not yet a routine part of the evaluation carried out by RHIF.

Due to the limitation of financial resources, there are several rules that should be followed: first-class drugs representing new mechanisms of action must demonstrate superior efficacy/safety and must not cost more than the lowest published wholesale price in the reference countries.

New drugs within an existing therapeutic class can be added to the List if the assessment shows no impact on the existing budget. Generics, depending on the order of entry, reduce the price (10 to 30 percent) of already mentioned drugs with the same inter-republic unprotected name (IGN). This way, the expansion of the list of medicines, provided that there is no impact on the budget, has been limited mainly to generic medicines for years, which has seriously affected access to innovative medicines (given that 22 innovative medicines entered the List thanks to managed entry agreements in 2016, only several of them were added in the next three years) [5].

However, due to the given rights, even drugs that are not on the list of drugs, but are necessary for treatment, under the conditions prescribed by the RHIF Law (Articles 15, 16 and 17 of the Rulebook on the Content and Scope of Rights) up to health care from compulsory health insurance and participation are provided to the insured person [10]. It refers to drugs that are not on the list of drugs, but are proven to be effective for certain indications in a specific patient when all other treatment options have already been applied without a positive result, it also refers to rare diseases under similar conditions of proven effectiveness, and the third case when the drug must ensure her status after transplantation abroad.

In a situation of slow progress towards the introduction of innovative medicines on the Medicines List, these three additional options significantly improve access to essential medicines.

The role of voluntary health insurance (VHI)

The Republic Health Insurance Fund (RHIF) implements voluntary health insurance with the aim of enabling citizens, under the most favorable conditions, to secure rights that are not covered by mandatory health insurance.

Regulation on health care Article 10 explains the process of obtaining a license from the National Bank for the performance of voluntary health insurance [11].

According to Article 30 of the aforementioned regulations, the following types of voluntary health insurance exist in Serbia:

- **Parallel health insurance** is insurance to cover health care costs incurred when the insured person obtains health care covered by mandatory health insurance in a manner and procedure different from the manner and procedure prescribed by law and regulations adopted for the implementation of the law on insurance.

This insurance is most often used for the purpose of using the insured's health care during their stay abroad. The National Bank provides data on consumption in Serbia for these purposes;

- **Supplementary health insurance** is insurance that covers costs that are not covered by rights from mandatory health insurance, and refer to health services, medicines, medical and technical aids, implants, coverage of greater content, scope and standards of rights;
- **Private health insurance** as insurance for persons who are not covered by mandatory health insurance or were not included in mandatory health insurance, to cover costs for the type, content, scope and standard of rights contracted with the insurance provider.

- Informal payments

Informal payment of health services in public institutions of Serbia is prohibited by law for both providers and recipients of such payments. However, informal payments for health services, especially in hospitals, are increasing [1]. Exceptions are the expression of gratitude as a gift of lesser value or advertising material and samples, which are not expressed in money.

Expressing gratitude in the form of a gift whose value does not exceed 5%, and the total value does not exceed the amount of one average monthly salary in the Republic of Serbia without taxes and contributions, is not considered corruption, conflict of interest, or private interest in accordance with the Law on Health Care [6].

DISCUSSION

The main gaps in the financial coverage of healthcare in the observed period, according to data from the National Health Account, refer to:

– Cancer therapy, orthopedic and heart surgeries, which are often paid for out of pocket by those who could not wait on waiting lists and could afford it.

Also, medicines, dental, laboratory and diagnostic services are obtained mostly from one's own pocket;

– Limited health care provided by Voluntary Health Insurance leads people to spend unplanned on health services;
– Informal payments for inpatient care are frequent, which makes a difference in the status of those who can afford it and those who are unable to pay for the service;
– There is a problem with waiting lists that are legally overcome by personal financing for a better position on the list, a greater financial burden for the poor without the possibility to provide a better place on the list with personal finances.

Concerns about financial protection in healthcare remained throughout the reform period of the healthcare system. Over the years, households have been exposed to a growing burden of out-of-pocket payments, so they have increasingly avoided using health services.

In particular, cancer patients are at high risk of impoverishment, as financial barriers to paying for prescribed diagnostic services, treatments, and medications have increased. The population with cancer often decided to sell their property to pay for expensive treatments and operations.

CONCLUSION

An overview of the reforms that took place in the period from 2004 to 2020 in the health sector of the Republic of Serbia and its financing forms an important basis for planning the functioning of health care and the implementation of subsequent reforms.

The general conclusion of the analysis is that in the observed period, Serbia had a good system of exemption from co-payments, but that out-of-pocket payments for certain health services and corrupt payments represented a barrier to health care.

RECOMMENDATION

It is necessary to make more efforts during the health care reform and its financing, in order to overcome the observed problems in the observed period from 2004 to 2020 and to remove financial barriers to the use of health care.

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Received: 9.3.2023 • Accepted: 30.5.2023

Pregled promena u zdravstvenom sektoru i njegovom finansiranju u Republici Srbiji u periodu od 2004. do 2020. godine

Milena Gajić-Stevanović, Ivan Stevanović

NVO Zdravstveni monitor, Beograd, Srbija

KRATAK SADRŽAJ

Uvod U periodu od 2004. do 2020. godine u zdravstvenom sektoru Republike Srbije i njegovom finansiranju sprovedene su mnoge reforme.

Cilj ovog rada je bio da se izvrši uvid u osnove na kojima se baziralo zdravstvo u Republici Srbiji u periodu od 2004. do 2020. godine i pregledaju izvršene reformske promene u zdravstvenom sektoru i njegovom finansiranju.

Materijal i metode Urađena je retrospektivna analiza podataka iz Nacionalnog zdravstvenog računa Instituta za javno zdravlje Srbije, pravilnika Republičkog fonda za zdravstveno osiguranje, izveštaja Ministarstva zdravlja, kao i zakona i uredbi vezanih za zdravstveni sektor.

Rezultati Rezultati analize su pokazali da je državni zdravstveni sektor predstavljao temelj zdravstvenog sistema u Republici Srbiji u posmatranom periodu. Od ukupnog finansiranja zdravstva, procenjeno je da su oko šezdeset posto činile šeme javnog finansiranja, a da je oko četrdeset posto pripadalo privatnim šemama, pri čemu je veliki deo privatnih šema činilo plaćanje iz džepa.

Zaključak Opšti zaključak analize je da je u posmatranom periodu Srbija u okviru reformskih promena imala dobar sistem oslobađanja od participacije, ali da su plaćanja iz džepa za određene zdravstvene usluge i koruptivna plaćanja predstavljala barijeru ka zdravstvenoj zaštiti.

Ključne reči: reforma u zdravstvenom sektoru; finansiranje zdravstvene zaštite; bazični paket zdravstvenih usluga

UVOD

Sistem zdravstvene zaštite Republike Srbije zasniva se na principima obaveznog zdravstvenog osiguranja. Republički fond za zdravstveno osiguranje (RFZO) predstavlja nacionalnu organizaciju kojom građani ostvaruju pravo na zdravstveno osiguranje i finansiraju zdravstvenu zaštitu.

RFZO finansira funkcionisanje zdravstvene zaštite na svim nivoima, ugovara pružanje usluga sa javnim i privatnim zdravstvenim ustanovama, kontroliše sprovođenje obaveza preuzetih prilikom ugovaranja sa njima, definiše osnovni paket zdravstvenih usluga.

Zbog nepostojanja adekvatne mreže privatnog zdravstvenog osiguranja, privatno finansiranje zdravstva je uglavnom zasnovano na plaćanju iz džepa, a dopunjeno je učešćem malog broja velikih kompanija.

Pomenute kompanije imaju (i finansiraju) svoje zdravstvene ustanove koje su specijalizovane za lečenje profesionalnih oboljenja, a pružaju i sve usluge iz domena primarne zdravstvene zaštite.

Cilj ovog rada je bio da se izvrši uvid u osnove na kojima se baziralo zdravstvo u Republici Srbiji u periodu od 2004. do 2020. godine i pregledaju izvršene reformske promene u zdravstvenom sektoru i njegovom finansiranju.

METODOLOGIJA I MATERIJAL

Urađena je retrospektivna analiza podataka iz Nacionalnog zdravstvenog računa Instituta za javno zdravlje Srbije, pravilnika Republičkog fonda za zdravstveno osiguranje, izveštaja Ministarstva zdravlja, kao i zakona i uredbi vezanih za zdravstveni sektor.

REZULTATI

U periodu od 2004. do 2020. godine, od ukupnog finansiranja zdravstva, procenjeno je da su oko šezdeset posto činile šeme javnog finansiranja, a da je oko četrdeset posto pripadalo privatnim šemama, pri čemu je veliki deo privatnih šema činilo plaćanje iz džepa [1]. Oko 1,5% od 60% šema javnog finansiranja se usmeravalo na finansiranje privatnih pružalaca zdravstvenih usluga, a ostatak na javne pružaoce usluga. Od 40% privatnih finansijskih šema, procenjeno je da se 35% usmeravalo ka privatnim pružaocima zdravstvenih usluga, a 5% ka javnim pružaocima usluga.

U okviru javnog sektora finansijera zdravstvene zaštite u Republici Srbiji predominantni finansijer je bio Republički fond za zdravstveno osiguranje (RFZO) sa učešćem oko 94% [1]. RFZO se finansirao iz doprinosa zaposlenih i poslodavaca u visini od 67%, 22% iz Fonda PIO, 4% iz doprinosa samozaposlenih, a 7% iz državnih transfera na ime posebnih ugroženih grupa stanovništva [1].

Javna zdravstvena zaštita se direktno pružala preko mreže zdravstvenih ustanova definisanih Uredbom o Planu mreže zdravstvenih ustanova [2]. Po planu mreže u 2020. godini bilo je 313 zdravstvenih ustanova (bez ustanova sa Kosova i Metohije) na primarnom, sekundarnom i tercijarnom nivou.

U posmatranom periodu Ministarstvo zdravlja poseduje većinu zdravstvenih ustanova i direktno ih finansira uglavnom kroz kapitalna ulaganja. Ministarstvo finansija prenosi sredstva RFZO-u za obezbeđenje zdravstvene zaštite ugroženih neosiguranih grupa: dugotrajno nezaposlenih, neosiguranih starijih i veoma mladih ljudi, kao i migranata, izbeglica i interno raseljenih lica.

Donacije stranih zemalja i međunarodnih organizacija se takođe transferišu u RFZO. Lokalna uprava doprinosi finansiranju zdravstva, uglavnom u investicione svrhe.

Model plaćanja za primarnu i sekundarnu zdravstvenu zaštitu

Zdravstvena reforma između 2004. i 2020. godine reformisala je zdravstvenu službu i fokusirala se na primarnu zdravstvenu zaštitu i prevenciju u odnosu na kurativnu zaštitu, kako bi se smanjila stopa izlečivih bolesti i zdravstveni izdaci.

Kao model plaćanja za primarnu zdravstvenu zaštitu izabrana je kapitacija (primena formule kapitacije počela je u novembru 2012. godine u ustanovama primarne zdravstvene zaštite) i model dijagnostički srodnih grupa (DSG) za plaćanja u sekundarnoj zdravstvenoj zaštiti.

Došlo je do ključnih promena u kapitacionoj formuli u 2020. godini utvrđenoj Uredbom o korektivnom koeficijentu u kapitacionoj formuli [3]. Pomenutom Uredbom su pored korektivnih faktora kao što je starost pacijenta, korektivnog faktora za gustinu naseljenosti, specifičnih indikatora kvaliteta za svaku specijalnost, uvedeni i korektivni faktori efikasnosti i dijagnostičko-terapijski postupak (DTP) kao korektivni faktori za sve specijalnosti.

Kod dijagnostički srodnih grupa promene se dešavaju u načinu bolničkog izveštavanja i u modeliranju budžeta. Postepeno se prelazi sa retrospektivnog načina plaćanja zdravstvenih usluga, koji je bio zasnovan na istorijskom budžetu, na prospektivni sistem plaćanja zasnovan na dijagnostički srodnim grupama.

Zakoni koji regulišu zdravstvenu zaštitu

Pružanje zdravstvene zaštite u Republici Srbiji uređuje se: Zakonom o zdravstvenoj zaštiti, Zakonom o zdravstvenom osiguranju i Zakonom o javnom zdravlju.

Ustav Republike Srbije definiše Republiku Srbiju kao državu srpskog naroda i svih njenih građana, utvrđuje vladavinu prava i socijalne pravde, principe građanske demokratije, ljudska i manjinska prava i slobode, kao i pripadnost evropskim principima i vrednostima [4].

Prema članu 68 Ustava svako ima pravo na zaštitu svog fizičkog i psihičkog zdravlja po principu solidarnosti u finansiranju i principu jednakosti u pristupu zdravstvenoj zaštiti.

Obavezno zdravstveno osiguranje je organizovano na principu: obaveznosti, solidarnosti i reciprociteta, zaštite prava osiguranika i zaštite javnog interesa, kontinuiranog unapređenja kvaliteta obaveznog zdravstvenog osiguranja i ekonomičnosti, kao i efikasnosti obaveznog zdravstvenog osiguranja [5].

Načelo obaveznog osiguranja odnosi se na obavezu plaćanja doprinosa za obavezno zdravstveno osiguranje po osnovu 12,3% do 2014. godine i od tada do danas 10,3% odbitka od plate [6].

Bez obzira na visinu raspoloživih sredstava i fluktuiranja troškova zdravstvene zaštite u posmatranom periodu, Zakon o zdravstvenoj zaštiti i Zakon o zdravstvenom osiguranju definišu osnovni paket zdravstvenih usluga. Prema ovim zakonima, obavezno zdravstveno osiguranje [3] pokriva (A) bolesti i povrede koje nisu povezane sa radom i (B) povrede i bolesti u vezi sa radom.

Pored toga, sredstvima obaveznog zdravstvenog osiguranja pokrivaju se troškovi rehabilitacije, nabavke medicinsko-tehničkih sredstava, lekova na recept, bolovanja (duže od mesec dana), troškovi prevoza u vezi sa korišćenjem zdravstvene zaštite i dr. Bolovanje do 30 dana pokriva poslodavac i o njima nema redovno prikupljanih podataka od strane države.

Društvena briga o zdravlju ostvarena je pružanjem zdravstvene zaštite grupama stanovništva sa povećanim rizikom od oboljenja, zdravstvenom zaštitom lica u vezi sa prevencijom, kontrolom, ranim otkrivanjem i lečenjem bolesti i stanja od većeg javnozdravstvenog značaja, kao i zdravstvenom zaštitom socijalno ugroženog stanovništva, pod jednakim pravima na teritoriji Republike Srbije [7].

Bazični paket zdravstvenih usluga predstavlja usluge koje se obezbeđuju iz sredstava obaveznog zdravstvenog osiguranja, prema članu 52 Zakona o zdravstvenom osiguranju, nomenklaturi (naziv i opis) zdravstvenih usluga i cenovniku zdravstvenih usluga iz obaveznog zdravstvenog osiguranja. Bazični paket su činili [5]:

1. mere za prevenciju i rano otkrivanje bolesti;
2. pregledi i lečenje u vezi sa planiranjem porodice, tokom trudnoće, porođaja i do 12 meseci nakon porođaja;
3. pregledi i lečenje u slučaju bolesti i povrede;
4. pregledi i lečenje bolesti usta i zuba;
5. medicinska rehabilitacija u slučaju bolesti i povrede;
6. lekovi;
7. medicinska sredstva.

Prema Zakonu o zdravstvenom osiguranju [5] osiguranici su u potpunosti pokriveni bez ikakve participacije za: mere prevencije i ranog otkrivanja bolesti, skrining i lečenje za planiranje porodice, trudnoću, porođaj i postporođajnu negu, uključujući prekid trudnoće iz medicinskih razloga, preglede, lečenje i medicinsku rehabilitaciju u slučaju bolesti i povreda dece, učenika i učenika do završetka propisanog školovanja, a najkasnije do 26. godine, odnosno starijih lica sa teškim fizičkim ili psihičkim smetnjama, preglede i lečenje usta i bolesti zuba u vezi sa trudnoćom i 12 meseci nakon porođaja, preglede i lečenje zaraznih bolesti koje su po zakonu obavezne za sprovođenje mera za sprečavanje njihovog širenja, preglede i lečenje maligniteta, dijabetesa, psihoza, epilepsije, multiple skleroze, progresivnih neuro-mišićnih oboljenja, cerebralne paralize, paraplegije, tetraplegije, trajne hronične bubrežne insuficijencije koja ukazuje na dijalizu ili transplantaciju bubrega, sistemskih autoimunih bolesti, reumatskih bolesti i njihovih komplikacija, retkih bolesti, palijativno zbrinjavanje, preglede i lečenje u vezi sa uzimanjem, primenom i razmenom organa, ćelija i tkiva za transplantaciju, za pružanje zdravstvene zaštite osiguranim licima, preglede, lečenje i rehabilitaciju od profesionalnih oboljenja i povreda na radu, pružanje hitne medicinske i stomatološke pomoći, kao i hitan medicinski prevoz.

Ako se ovako utvrđen sadržaj i obim prava na zdravstvenu zaštitu iz obaveznog zdravstvenog osiguranja ne može ostvariti zbog nedovoljnih sredstava, Vlada donosi akt kojim se utvrđuju prioriteti u pružanju i sprovođenju zdravstvene zaštite.

Takođe, Vlada svake godine, na predlog ministra zdravlja, donosi akt kojim se utvrđuju prioriteti u lečenju pacijenata sa određenim vrstama retkih bolesti, za šta su obezbeđena sredstva u budžetu Republike Srbije.

Pokrivenost uz participaciju se procentualno razlikuje zavisno od vrste zdravstvene usluge:

– U iznosu od najmanje 95% cene zdravstvene usluge iz fondova obaveznog zdravstvenog osiguranja za: intenzivnu negu u stacionarnoj zdravstvenoj ustanovi, hirurški zahvat

koji se obavlja u operacionoj sali, uključujući ugradnju implantata za najsloženije i najskuplje zdravstvene usluge, najsloženije laboratorijske, rendgenske i druge dijagnostičke i terapijske procedure (magnetna rezonanca, skener, nuklearna medicina i dr.);
- U iznosu od najmanje 80% cene zdravstvene usluge iz sredstava obaveznog zdravstvenog osiguranja za: preglede i lečenje kod izabranog lekara i lekara specijaliste, laboratorijske, rendgenske i druge dijagnostičke i terapijske procedure, kućno lečenje, stomatološke preglede i lečenje u vezi sa povredama zuba i kostiju lica, kao i stomatološke preglede i stomatološke tretmane pre operacije srca i transplantacije organa, ćelija i tkiva. Takođe za lečenje komplikacija karijesa kod dece, studenata i studenata do isteka propisanog roka školovanja, a najkasnije do 26. godine, vađenje zuba kao posledicu karijesa, kao i razvoj mobilnih ortodontskih aparata, stacionarno lečenje, kao i rehabilitaciju u stacionarnoj zdravstvenoj ustanovi, preglede i lečenje u dnevnoj bolnici, uključujući hirurgiju van operacione sale, ambulantnu medicinsku rehabilitaciju, neka medicinska sredstva.
- U iznosu od najmanje 65% cene zdravstvene usluge iz fondova obaveznog zdravstvenog osiguranja za: izradu akrilatnih totalnih i subtotalnih proteza kod lica starijih od 65 godina, očna i slušna pomagala za odrasle, promenu pola iz medicinskih razloga, medicinski transport koji nije hitan, lečenje bolesti čije rano otkrivanje podleže ciljanom preventivnom skriningu, odnosno skriningu u skladu sa relevantnim nacionalnim programima, ako se osigurano lice nije odazvalo ni na jedan poziv u okviru jednog ciklusa poziva, ili opravdalo izostanak, a dijagnostikovano mu je oboljenje do sledećeg ciklusa poziva.

Zdravstvene usluge koje nisu opisane u bazičnom paketu usluga čine usluge koje osigurano lice plaća iz sopstvenih sredstava, po cenama koje odredi davalac zdravstvene zaštite.

Negativna lista zdravstvenih usluga predstavlja listu intervencija, usluga i proizvoda koji se uglavnom plaćaju iz džepa u javnim ili privatnim zdravstvenim ustanovama, a ne nalaze se na listi bazičnog paketa usluga.

Novi Zakon o zdravstvenom osiguranju koji je stupio na snagu 2019. godine omogućio je više prava za korišćenje zdravstvenih usluga, poput promene pola iz medicinskih razloga i veštačke oplodnje za žene [5], u poređenju sa Zakonom iz 2005. godine.

Troškovi korisnika zdravstvene zaštite za lekove i pomoćna sredstva

Republički fond za zdravstveno osiguranje u svojim ugovorima sa javnim ustanovama unapred aproksimira participaciju koju svaka ustanova ostvaruje. Aproksimirana suma se umanjuje od transferisanih sredstava u ustanove. Visina aproksimirane participacije iznosi 1–3% od ukupnog prihoda zdravstvenih ustanova.

Država Srbija donosi podzakonske akte i propise kojima se uređuje obim prava na zdravstvenu zaštitu iz obaveznog zdravstvenog osiguranja, a u okviru kojih je obuhvaćeno i pravo na lekove. Troškovi stanovništva za lekove i pomoćna sredstva čine oko šezdeset posto [1] njihovih ukupnih direktnih troškova za zdravstvenu zaštitu, iako i RFZO ulaže značajna finansijska sredstva.

Prema pravilniku RFZO [8], na listi lekova koji se propisuju i izdaju po osnovu obaveznog zdravstvenog osiguranja, svi lekovi su podeljeni u pet osnovnih grupa:
1) A. Lekovi koji se propisuju i izdaju na recept (Lista A, lekovi koji se izdaju uz fiksnu participaciju – 50 dinara po receptu);
2) A1. Lekovi koji se propisuju i izdaju u obliku lekarskog recepta, koji imaju terapijsku paralelu (terapeutsku alternativu) lekovima sa Liste A (Lista A1, lekovi se izdaju uz 10 do 90% participacije po pakovanju);
3) B. Lekovi koji se koriste tokom ambulantnog ili bolničkog lečenja u zdravstvenim ustanovama (Lista B, lekovi koji se izdaju bez participacije);
4) C. Lekovi sa posebnim režimom (Lista C, lekovi koji se izdaju bez participacije);
5) lekovi koji nemaju dozvolu za stavljanje u promet u Republici Srbiji, ali su neophodni u dijagnostici i terapiji – neregistrovani lekovi, a izuzetno lekovi su registrovani u Republici Srbiji sa istim generičkim nazivom (IGN) kao lek koji se stavlja na listu lekova, a koja nije dostupna na tržištu Republike Srbije u količinama neophodnim za pružanje zdravstvene zaštite osiguranim licima, odnosno koja je povučena sa tržišta (Lista D, lekovi izdati bez participacije).

Ceo proces stavljanja leka ili njegovog brisanja sa liste lekova mora biti u skladu sa Pravilnikom o uslovima, kriterijumima, načinu i postupku za stavljanje leka na Listu lekova, izmenama i dopunama Liste lekova, odnosno za brisanje leka sa Liste lekova [9].

Ključne informacije koje zahtevaju tadašnji propisi su o dokazima o bezbednosti i efikasnosti, zajedno sa farmako-ekonomskom analizom zasnovanom na troškovima prema definisanoj dnevnoj dozi i analizom uticaja na budžet. Međutim, potrebna je analiza isplativosti koja još uvek nije rutinski deo procene koju vrši RFZO.

Zbog ograničenja finansijskih resursa, postoji nekoliko pravila koja bi trebalo da se poštuju: prvoklasni lekovi koji predstavljaju nove mehanizme delovanja moraju da pokažu superiornu efikasnost/bezbednost i ne smeju da imaju višu cenu od najniže objavljene veleprodajne cene u referentnim zemljama.

Novi lekovi u okviru postojeće terapijske klase mogu se dodati na Listu ako procena ne pokaže uticaj na postojeći budžet. Generici, u zavisnosti od redosleda unosa, snižavaju cenu (10 do 30 odsto) već navedenih lekova sa istim međurepubličkim nezaštićenim nazivom (IGN). Na taj način proširenje liste lekova, pod uslovom da nema uticaja na budžet, godinama je ograničeno uglavnom na generičke lekove, što je ozbiljno uticalo na pristup inovativnim lekovima (s obzirom na to da su 22 inovativna leka ušla na Listu zahvaljujući ugovorima o upravljanom ulasku u 2016, samo nekoliko njih [5] dodato je u naredne tri godine). Međutim, zbog datih prava, čak i lekovi koji nisu na listi lekova ali su neophodni za lečenje, pod uslovima propisanim Zakonom RFZO (čl. 15, 16. i 17. Pravilnika o sadržini i obimu prava) do zdravstvene zaštite od obaveznog zdravstvenog osiguranja i participacije (10)) obezbeđuju se osiguranom licu. Odnosi se na lekove koji nisu na Listi lekova, ali su dokazano delotvorni za određene indikacije kod određenog pacijenta kada su sve druge opcije lečenja već primenjene bez pozitivnog rezultata, odnosi se i na retke bolesti pod sličnim uslovima dokazane efikasnosti,

a treći slučaj kada se lek mora obezbediti je status nakon transplantacije u inostranstvu.

U situaciji usporenog kretanja ka uvođenju inovativnih lekova na Listu lekova, ove tri dodatne opcije značajno poboljšavaju pristup neophodnim lekovima.

Uloga dobrovoljnog zdravstvenog osiguranja (DZO)

RFZO sprovodi dobrovoljno zdravstveno osiguranje sa ciljem da građanima omogući da, pod najpovoljnijim uslovima, obezbede prava koja nisu obuhvaćena obaveznim zdravstvenim osiguranjem.

Uredba o zdravstvenoj zaštiti [11], član 10, objašnjava proces dobijanja dozvole od Narodne banke za obavljanje poslova dobrovoljnog zdravstvenog osiguranja.

Prema članu 30 navedenih propisa, u Srbiji postoje sledeće vrste dobrovoljnog zdravstvenog osiguranja:

– **Paralelno zdravstveno osiguranje** je osiguranje za pokrivanje troškova zdravstvene zaštite nastalih kada osigurano lice ostvari zdravstvenu zaštitu obuhvaćenu obaveznim zdravstvenim osiguranjem na način i postupak drugačiji od načina i postupka propisanog zakonom i propisima donetim za sprovođenje zakona o osiguranju.

Ovo osiguranje se najčešće koristi u svrhu korišćenja zdravstvene zaštite osiguranika tokom boravka u inostranstvu. Narodna banka daje podatke o potrošnji u Srbiji za te namene.

– **Dopunsko zdravstveno osiguranje** je osiguranje koje pokriva troškove koji nisu obuhvaćeni pravima iz obaveznog zdravstvenog osiguranja, a odnose se na zdravstvene usluge, lekove, medicinska i tehnička pomagala, implantate, pokriće većeg sadržaja, obima i standarda prava.
– **Privatno zdravstveno osiguranje** je osiguranje lica koja nisu obuhvaćena obaveznim zdravstvenim osiguranjem ili nisu bila uključena u obavezno zdravstveno osiguranje. Pokriva troškove za vrstu, sadržaj, obim i standard prava ugovorenih sa davaocem osiguranja.

Neformalna plaćanja

Neformalno plaćanje zdravstvenih usluga u javnim ustanovama Srbije je zakonom zabranjeno i za pružaoce i za primaoce takvih plaćanja. Međutim, neformalna plaćanja za zdravstvene usluge, posebno u bolnicama, rastu [1]. Izuzeti su iskazivanje zahvalnosti kao poklon manje vrednosti ili reklamnog materijala i uzoraka, koji nisu izraženi u novcu.

Iskazivanje zahvalnosti u vidu poklona čija vrednost ne prelazi 5%, a ukupna vrednost ne prelazi iznos jedne prosečne mesečne plate u Republici Srbiji bez poreza i doprinosa, ne smatra se korupcijom, sukobom interesa, odnosno privatnim interesom u skladu sa Zakonom o zdravstvenoj zaštiti [6].

DISKUSIJA

Glavne praznine u finansijskoj pokrivenosti zdravstva u posmatranom periodu, prema podacima iz Nacionalnog zdravstvenog računa, odnose se na sledeće:

– Terapija raka, ortopedske i srčane operacije često se plaćaju iz džepa onih koji nisu mogli da čekaju na listama čekanja, a mogli su da to sebi priušte. Takođe, lekovi, stomatološke, laboratorijske i dijagnostičke usluge dobijaju se uglavnom iz sopstvenog džepa.
– Ograničena zdravstvena zaštita koju pruža Dobrovoljno zdravstveno osiguranje dovodi ljude u situaciju da troše neplanski na zdravstvene usluge.
– Česta su neformalna plaćanja za stacionarnu negu, što pravi razliku u statusu onih koji to mogu sebi da priušte i onih koji nisu u mogućnosti da plate uslugu.
– Postoji problem sa listama čekanja koje se zakonski prevazilaze ličnim finansiranjem za bolju poziciju na listi. Veće je finansijsko opterećenje za siromašnije, bez mogućnosti da ličnim finansijama obezbede bolje mesto na listi.

Zabrinutost oko finansijske zaštite u zdravstvu ostala je tokom čitavog reformskog perioda zdravstvenog sistema. Tokom godina domaćinstva su bila izložena rastućem teretu plaćanja iz džepa, tako da su sve više izbegavala korišćenje zdravstvenih usluga.

Stanovništvo se često odlučuivalo na prodaju imovine da bi platili skupa lečenja i operacije. Konkretno, pacijenti sa kancerom su u visokom riziku od osiromašenja, jer su se povećale finansijske prepreke za plaćanje propisanih dijagnostičkih usluga, tretmana i lekova.

ZAKLJUČAK

Pregled reformi koje su se dogodile u periodu od 2004. do 2020. godine u u zdravstvenom sektoru Republike Srbije i njegovom finansiranju čini značajnu osnovu za planiranje funkcionisanja zdravstvene zaštite i sprovođenje narednih reformi.

Opšti zaključak analize je da je u posmatranom periodu Srbija imala dobar sistem oslobađanja od participacije, ali da su plaćanja iz džepa za određene zdravstvene usluge i koruptivna plaćanja predstavljala barijeru ka zdravstvenoj zaštiti.

PREPORUKA

Potrebno je uložiti više napora tokom reforme zdravstvene zaštite i njenog finansiranja, kako bi se uočeni problemi u posmatranom periodu od 2004. do 2020. godine prevazišli i kako bi nestale finansijske barijere kod korišćenja zdravstvene zaštite.

Da li ste pažljivo čitali radove?

1. Određivanje debljine dentina urađeno je:
   a) na bukomezijalnom korenu donjeg molara
   b) na bukomezijalnom korenu gornjeg molara
   c) na bukomezijalnom korenu gornjeg umnjaka

2. Uticaj irigacije je proveravan:
   a) kod opturacije kanala
   b) kod restauracije zuba
   c) kod aplikacije kočića

3. Marginalna mikropropustljivost je proveravana kod:
   a) fosfatnog cementa
   b) kalcijum-aluminatnog cementa
   c) magnezijum-fosfatnog cementa

4. Gram-negativne bakterije obuhvataju:
   a) više od 10 rodova
   a) više od 15 rodova
   b) više od 20 rodova

5. U periodu 2004–2020 u zdravstvenom sektoru Republike Srbije:
   a) sprovedeno je mnogo reformi
   b) nije bilo reformi
   c) urađene su samo dve reforme

6. Provera jačine veze kompozitnih kočića za dentin urađena je:
   a) metodom prodora boje
   b) push-out testom
   c) testom na smicanje

7. Veza kompozitnih kočića je proveravana:
   a) primenom jedne adhezivne tehnike
   b) primenom dve adhezivne tehnike
   c) primenom tri adhezivne tehnike

8. Najmanja debljina zidova na donjem molaru se nalazi:
   a) na 1 mm ispod furkacije
   b) na 2-3 mm ispod furkacije
   c) na 5-6 mm ispod furkacije

9. Kalcijum-aluminatni cementi su aplikovani u:
   a) apikalne perforacije
   b) interradiksne perforacije
   c) gingivalne perforacije

10. Najčešće izolovan rod Gram-negativnih bakterija je:
    a) *Bacteroides* spp.
    b) *Tannerella* spp.
    c) *Treponema* spp.

11. Od ukupnog finansiranja zdravstvenog sistema u Srbiji, šeme javnog finansiranja obuhvatile su:
    a) 60%
    b) 40%
    c) 39%

12. U eksperimentu o uticaju irigacije na kvalitet veze kompozitnih kočića korišćen je:
    a) 1% rastvor NaOCl
    b) 2,5% rastvor NaOCl
    c) 5,25% rastvor NaOCl

13. Adhezivna veza je proveravana kod:
    a) kompozitnih kočića
    b) metalnih kočića
    c) cementnih kočića

14. Debljina dentina merena je na udaljenosti od furkacije korena:
    a) 5 mm
    b) 6 mm
    c) 8 mm

15. Kvalitet rubnog zaptivanja kalcijum-aluminatnih sistema je proveravan:
    a) metodom prodora boje
    b) metodom prodora bakterija
    c) metodom prodora proteina

16. Inkubacija anaerobnih bakterija je:
    a) kraća u odnosu na aerobne bakterije
    b) duža u odnosu na aerobne bakterije
    c) identična sa aerobnim bakterijama

17. Fusobacterium i Veillonella se javljaju kod nekih osoba:
    a) sa HIV-om
    b) sa karcinomom pluća
    c) sa koštano-zglobnim oboljenjima

18. Šeme privatnog finansiranja u zdravstvenom sektoru Srbije čine:
    a) 40%
    b) 50%
    c) 60%

19. Određivanje debljine dentina realizovano je:
    a) rendgenografijom
    b) metodom CBCT
    c) merenjem lenjirom

20. Provera adhezivne veze kod kompozitnih kočića je urađena:
    a) kod 28 jednokorenih zuba
    b) kod 38 jednokorenih zuba
    c) kod 48 jednokorenih zuba

21. Kao kontrolni materijal u proveri kvaliteta rubnog zatvora kod interradiksnih perforacija korišćen je:
    a) fosfatni cement
    b) MTA
    c) biodentin

22. Marginalna propustljivost novosintetisanih kalcijum-aluminata je realizovana u:
    a) Beogradu
    b) Foči
    c) Banjoj Luci

23. P. endodontalis se uglavnom izoluje iz:
    a) karijesnih lezija
    b) inficiranih kanala
    c) resorptivnih lakuna

24. Reformske promene u zdravstvenom sistemu Srbije su obezbedile:
    a) dobar sistem oslobađanja od participacije
    b) loš sistem oslobađanja od participacije
    c) barijeru za oslobađanje od participacije

25. Merenje debljine dentina distalnog zida mezijalnog korena prvog donjeg molara urađeno je kod:
    a) stanovnika Banje Luke
    b) stanovnika Foče
    c) stanovnika Mostara

26. Adhezivna veza kompozitnog kočića je proveravana kod:
    a) jednokorenih zuba
    b) dvokorenih zuba
    c) višekorenih zuba

27. Za merenje debljine dentina donjih molara korišćeni su:
    a) aksijalni preseci
    b) poprečni preseci
    c) kombinacija aksijalnih i poprečnih preseka

28. Ispitivanje kvaliteta rubnog zatvaranja je realizovano kod:
    a) 38 molara
    b) 48 molara
    c) 58 molara

29. Mikropropustljivost materijala na bazi kalcijum-aluminata je bila:
    a) veća u odnosu na kalcijum-silikate
    b) veća u odnosu na MTA
    c) značajno veća u odnosu na kalcijum-silikate i MTA

30. U okviru roda Prevotella opisano je:
    a) 25 vrsta
    b) 38 vrsta
    c) 49 vrsta

31. Barijeru za zdravstvenu zaštitu predstavljalo je:
    a) plaćanje iz džepa određenih zdravstvenih usluga
    b) korupcija
    c) plaćanje iz džepa i korupcija

32. Incidenca endodontskog tretmana kod prvog donjeg molara iznosi:
    a) 10%
    b) 15%
    c) 17%

33. Zubi u eksperimentu sa kočićima su podeljeni u:
    a) dve grupe
    b) tri grupe
    c) četiri grupe

34. Prema dužini korena donjeg molara zubi su klasifikovani:
    a) u dve grupe
    b) u tri grupe
    c) u četiri grupe

35. Marginalna propustljivost testiranog materijala je proveravana posle:
    a) dva meseca
    b) tri meseca
    c) šest meseci

36. Prosečan prodor boje kalcijum-silikata je iznosio:
    a) 1,40 mm
    b) 2,10 mm
    c) 1,73 mm

37. Bakterije iz roda Fusobacterium su povezane sa:
    a) karcinomom debelog creva
    b) karcinomom prostate
    c) karcinomom materice

38. Osnovu u analizi reformi zdravstvenog finansiranja u Srbiji činila je:
    a) analiza podrške iz Nacionalnog zdravstvenog računa
    b) analiza podrške iz Svetske zdravstvene organizacije
    c) analiza Evropskih fondova

39. U radu sa proverom debljine dentina na donjem molaru:
    a) ukupno je pregledano 372 CBCT snimka
    b) ukupno je pregledano 100 CBCT snimaka
    c) ukupno je pregledano 58 CBCT snimaka

40. Ispitivanja za proveru debljine dentina na donjem molaru su podeljena:
    a) u dve starosne grupe
    b) u tri starosne grupe
    c) u četiri starosne grupe

41. U terapiji infekcija uzrokovanih Gram-negativnim bakterijama koristi se:
    a) kombinacija antibiotika
    b) samo jedan antibiotik
    c) kombinacija analgetika

42. Reformske promene u zdravstvenom sistemu Srbije su analizirane u periodu:
    a) 2000–2020
    b) 2004–2020
    c) 2010–2022

43. Prevotella spp. izaziva oboljenja parodoncijuma?
    a) Da
    b) Ne
    c) Samo u izuzetnim slučajevima

44. Najmanji prosečni prodor boje je uočen kod:
    a) MTA
    b) kalcijum-silikata
    c) kalcijum-aluminata

45. Pri proveri debljine dentina donjeg molara od ukupno pregledanih CBCT snimaka izdvojeno je:
    a) 100 preseka zuba
    b) 58 preseka zuba
    c) 40 preseka zuba

46. Protokol irigacije utiče na jačinu veze kompozitnih kočiča?
    a) Da
    b) Ne
    c) Samo kod višekorenih zuba

47. Najveći prosečni prodor boje je uočen kod:
    a) kalcijum-silikata
    b) kalcijum-aluminata
    c) MTA

48. Prosečan prodor boje kalcijum-aluminata je iznosio:
    a) 1,40 mm
    b) 2,10 mm
    c) 1,73 mm

49. Prosečni prodor boje kod MTA je iznosio:
    a) 1,40 mm
    b) 2,10 mm
    c) 3,10 mm

50. Eksperiment o uticaju irigacije na kvalitet veze kompozitnih kočića realizovan je u:
    a) Sarajevu
    b) Skoplju
    c) Beogradu

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AUTORSTVO. Sve osobe koje su navedene kao autori rada treba da se kvalifikuju za autorstvo. Svaki autor treba da je učestvovao dovoljno u radu na rukopisu kako bi mogao da preuzme odgovornost za celokupan tekst i rezultate iznesene u radu. Autorstvo se zasniva samo na: bitnom doprinosu koncepciji rada, dobijanju rezultata ili analizi i tumačenju rezultata; planiranju rukopisa ili njegovoj kritičkoj reviziji od znatnog intelektualnog značaja; završnom doterivanju verzije rukopisa koji se priprema za štampanje.

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PLAGIJARIZAM. Od 1. januara 2019. godine svi rukopisi podvrgavaju se proveri na plagijarizam/autoplagijarizam preko SCIndeks Assistant – Cross Check (iThenticate). Radovi kod kojih se dokaže plagijarizam/autoplagijarizam biće odbijeni, a autori sankcionisani.

NASLOVNA STRANA. Na prvoj stranici rukopisa treba navesti sledeće: naslov rada bez skraćenica; predlog kratkog naslova rada, puna imena i prezimena autora (bez titula) indeksirana brojevima; zvaničan naziv ustanova u kojima autori rade, mesto i državu (redosledom koji odgovara indeksiranim brojevima autora); na dnu stranice navesti ime i prezime, adresu za kontakt, broj telefona, i imejl adresu autora zaduženog za korespondenciju.

SAŽETAK. Uz originalni rad, prethodno i kratko saopštenje, metaanalizu, pregled literature, prikaz slučaja (bolesnika), rad iz

istorije medicine, aktuelnu temu, rad za rubriku jezik medicine i rad za praksu, na drugoj po redu stranici dokumenta treba priloži ti sažetak rada obima 100–250 reči. Za originalne radove, prethodno i kratko saopštenje, metaanalize i pregledne radove, sažetak treba da ima sledeću strukturu: Uvod/Cilj, Metode, Rezultati, Zaključak; svaki od navedenih segmenata pisati kao poseban pasus koji počinje boldovanom reči. Navesti najvažnije rezultate (numeričke vrednosti) statističke analize i nivo značajnosti. Zaključak ne sme biti uopšten, već mora biti direktno povezan sa rezultatima rada. Za prikaze bolesnika sažetak treba da ima sledeće delove: Uvod (u poslednjoj rečenici navesti cilj), Prikaz bolesnika, Zaključak; segmente takođe pisati kao poseban pasus koji počinje boldovanom reči. Za ostale tipove radova sažetak nema posebnu strukturu.

KLJUČNE REČI. Ispod Sažetka navesti od tri do šest ključnih reči ili izraza. U izboru ključnih reči koristiti Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh).

PREVOD NA SRPSKI JEZIK. Na posebnoj stranici dokumenta priložiti naslov rada na srpskom jeziku, puna imena i prezimena autora (bez titula) indeksirana brojevima, zvaničan naziv ustanova u kojima autori rade, mesto i državu. Na sledećoj stranici dokumenta priložiti sažetak (100–250 reči) s ključnim rečima (3–6), prevod naziva priloga (tabela, grafikona, slika, shema) i celokupni tekst u njima i legendu.

STRUKTURA RADA. Svi podnaslovi se pišu velikim masnim slovima (bold). Originalni rad, metaanaliza, prethodno i kratko saopštenje obavezno treba da imaju sledeće podnaslove: Uvod (Cilj rada navesti kao poslednji pasus Uvoda), Metode rada, Rezultati, Diskusija, Zaključak, Literatura. Pregled literature čine: Uvod, odgovarajući podnaslovi, Zaključak, Literatura. Prvoimenovani autor metaanalize i preglednog rada mora da navede bar pet autocitata (kao autor ili koautor) radova publikovanih u časopisima s recenzijom. Koautori, ukoliko ih ima, moraju da navedu bar jedan autocitat radova takođe publikovanih u časopisima s recenzijom. Prikaz slučaja ili bolesnika čine: Uvod (Cilj rada navesti kao poslednji pasus Uvoda), Prikaz bolesnika, Diskusija, Literatura. Ne treba koristiti imena bolesnika, inicijale, niti brojeve istorija bolesti, naročito u ilustracijama. Prikazi bolesnika ne smeju imati više od pet autora. Priloge (tabele, grafikone, slike itd.) postaviti na kraj rukopisa, a u samom telu teksta jasno naznačiti mesto koje se odnosi na dati prilog. Krajnja pozicija priloga biće određena u toku pripreme rada za publikovanje.

SKRAĆENICE. Koristiti samo kada je neophodno, i to za veoma dugačke nazive hemijskih jedinjenja, odnosno nazive koji su kao skraćenice već prepoznatljivi (standardne skraćenice, kao npr. DNK, sida, HIV, ATP). Za svaku skraćenicu pun termin treba navesti pri prvom navođenju u tekstu, sem ako nije standardna jedinica mere. Ne koristiti skraćenice u naslovu. Izbegavati korišćenje skraćenica u sažetku, ali ako su neophodne, svaku skraćenicu objasniti pri prvom navođenju u tekstu.

DECIMALNI BROJEVI. U tekstu rada na engleskom jeziku, u tabelama, na grafikonima i drugim prilozima decimalne brojeve pisati sa tačkom (npr. 12.5 ± 3.8), a u tekstu na srpskom jeziku sa zarezom (npr. 12,5 ± 3,8). Kad god je to moguće, broj zaokružiti na jednu decimalu.

JEDINICE MERA. Dužinu, visinu, težinu i zapreminu izražavati u metričkim jedinicama (metar – m, kilogram (gram) – kg (g), litar – l) ili njihovim delovima. Temperaturu izražavati u stepenima Celzijusa (°C), količinu supstance u molima (mol), a pritisak krvi u milimetrima živinog stuba (mm Hg). Sve rezultate hematoloških, kliničkih i biohemijskih merenja navoditi u metričkom sistemu prema Međunarodnom sistemu jedinica (SI).

OBIM RADOVA. Celokupni rukopis rada – koji čine naslovna strana, sažetak, tekst rada, spisak literature, svi prilozi, odnosno potpisi za njih i legenda (tabele, slike, grafikoni, sheme, crteži), naslovna strana i sažetak na srpskom jeziku – mora iznositi za originalni rad, prethodno i kratko saopštenje, rad izistorije medicine i pregled literature do 5000 reči, a za prikaz bolesnika, rad za praksu, edukativni članak i rad za rubriku „Jezik medicine“ do 3000 reči; radovi za ostale rubrike mogu imati najviše 1500 reči.

PRILOZI RADU su tabele i slike (fotografije, crteži, sheme, grafikoni).

TABELE. Svaka tabela treba da bude sama po sebi lako razumljiva. Naslov treba otkucati iznad tabele, a objašnjenja ispod nje. Tabele se označavaju arapskim brojevima prema redosledu navođenja u tekstu. Tabele crtati isključivo u programu Word, kroz meni Table–Insert–Table, uz definisanje tačnog broja kolona i redova koji će činiti mrežu tabele. Desnim klikom na mišu – pomoću opcija Merge Cells i Split Cells – spajati, odnosno deliti ćelije. Kucati fontom Times New Roman, veličinom slova 12 pt, s jednostrukim proredom i bez uvlačenja teksta. Korišćene skraćenice u tabeli treba objasniti u legendi ispod tabele. Ukoliko je rukopis na srpskom jeziku, priložiti nazive tabela i legendu na oba jezika. Takođe, u jednu tabelu, u okviru iste ćelije, uneti i tekst na srpskom i tekst na engleskom jeziku (nikako ne praviti dve tabele sa dva jezika!).

SLIKE. Slike su svi oblici grafičkih priloga i kao „slike“ u SGS se objavljuju fotografije, crteži, sheme i grafikoni. Slike označavaju se arapskim brojevima prema redosledu navođenja u tekstu. Primaju se isključivo digitalne fotografije (crno-bele ili u boji) rezolucije najmanje 300 dpi i formata zapisa tiff ili jpg (male, mutne i slike lošeg kvaliteta neće se prihvatati za štampanje!). Ukoliko autori ne poseduju ili nisu u mogućnosti da dostave digitalne fotografije, onda originalne slike treba skenirati u rezoluciji 300 dpi i u originalnoj veličini. Ukoliko je rad neophodno ilustrovati sa više slika, u radu će ih biti objavljeno nekoliko, a ostale će biti u e-verziji članka kao PowerPoint prezentacija (svaka slika mora biti numerisana i imati legendu). Ukoliko je rukopis na srpskom jeziku, priložiti nazive slika i legendu na oba jezika.

GRAFIKONI. Grafikoni treba da budu urađeni i dostavljeni u programu Excel, da bi se videle prateće vrednosti raspoređene po ćelijama. Iste grafikone prekopirati i u Word-ov dokument, gde se grafikoni označavaju arapskim brojevima prema redosledu navođenja u tekstu. Svi podaci na grafikonu kucaju se u fontu Times New Roman. Korišćene skraćenice na grafikonu treba objasniti u legendi ispod grafikona. U štampanoj verziji članka verovatnije je da grafikon neće biti štampan u boji, te je bolje izbegavati korišćenje boja u grafikonima, ili ih koristiti

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SHEME (CRTEŽI). Crteži i sheme se dostavljaju u jpg ili tiff formatu. Sheme se mogu crtati i u programu CorelDraw ili Adobe Illustrator (programi za rad sa vektorima, krivama). Svi podaci na shemi kucaju se u fontu Times New Roman, veličina slova 10 pt. Korišćene skraćenice na shemi treba objasniti u legendi ispod sheme. Ukoliko je rukopis na srpskom jeziku, priložiti nazive shema i legendu na oba jezika.

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LITERATURA. Spisak referenci je odgovornost autora, a citirani članci treba da budu lako pristupačni čitaocima časopisa. Stoga uz svaku referencu obavezno treba navesti DOI broj članka (jedinstvenu nisku karaktera koja mu je dodeljena) i PMID broj ukoliko je članak indeksiran u bazi PubMed/MEDLINE. Reference numerisati rednim arapskim brojevima prema redosledu navođenja u tekstu. Broj referenci ne bi trebalo da bude veći od 30, osim u pregledu literature, u kojem je dozvoljeno da ih bude do 50, a u metaanalizi do 100. Broj citiranih originalnih radova mora biti najmanje 80% od ukupnog broja referenci, odnosno broj citiranih knjiga, poglavlja u knjigama i preglednih članaka manji od 20%. Ukoliko se domaće monografske publikacije i članci mogu uvrstiti u reference, autori su dužni da ih citiraju. Većina citiranih naučnih članaka ne bi trebalo dabude starija od pet godina. Nije dozvoljeno citiranje apstrakata. Reference članaka koji su prihvaćeni za štampu, ali još nisu objavljeni, treba označiti sa in press i priložiti dokaz o prihvatanju rada za objavljivanje.

Reference se citiraju prema Vankuverskom stilu (uniformisanim zahtevima za rukopise koji se predaju biomedicinskim časopisima), koji je uspostavio Međunarodni komitet urednika medicinskih časopisa (http://www.icmje.org), čiji format koriste U.S. National Library of Medicine i baze naučnih publikacija. Primeri navođenja publikacija (članaka, knjiga i drugih monografija, elektronskog, neobjavljenog i drugog objavljenog materijala) mogu se pronaći na internet-stranici http://www.nlm.nih.gov/bsd/uniform_requirements.html. Prilikom navođenja literature veoma je važno pridržavati se pomenutog standarda, jer je to jedan od najbitnijih faktora za indeksiranje prilikom klasifikacije naučnih časopisa.

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Za sve dodatne informacije, molimo da se obratite na dole navedene adrese i broj telefona:

ADRESA:
Srpsko lekarsko društvo
Uredništvo časopisa „Stomatološki glasnik Srbije“ Ul. kraljice Natalije 1
11000 Beograd Srbija
Telefon: + 381 (0)11 409-2776
Imejl adresa: stomglas@bvcom.rs
Internet adresa: http://www.stomglas.org.rs
ISSN Online 1452-3701
Open access

INSTRUCTIONS FOR AUTHORS

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CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

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KEYWORDS. Below the Summary, 3 to 6 keywords or phrases should be typed. The keywords need not repeat words in the title and should be relevant or descriptive. Medical Subject Headings – MeSH (http://www.nlm.nih. gov/mesh) are to be used for selection of the keywords.

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Serbian Dental Journal
Editorial Office of Serbian Dental Journal
Kraljice Natalije 1
11000 Belgrade
Serbia
Phone: (+381 11) 409-2776
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Website: www.stomglas.org.rs
ISSN Online 1452 3701
OPEN ACCESS

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

616.31

STOMATOLOŠKI glasnik Srbije = Serbian
Dental Journal / glavni i odgovorni urednik
Slavoljub Živković. - God. 1, br. 1 (1955)-
. - Beograd (Džordža Vašingtona 19) :
Srpsko lekarsko društvo, 1955- (Beograd :
Službeni glasnik). - 29,5 cm

Dostupno i na: http://www.stomglas.org.rs - Tromesečno

ISSN 0039-1743 = Stomatološki glasnik Srbije
(Štampano izd.)
COBISS.SR-ID 8417026