



# STOMATOLOŠKI GLASNIK SRBIJE

## SERBIAN DENTAL JOURNAL

Vol. 63 • Number 3 • July-September 2016





# STOMATOLOŠKI GLASNIK SRBIJE

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Vol. 63 • Number 3 • July-September 2016

**Adresa uredništva**  
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**Časopis izlazi četiri puta godišnje.**  
The journal is published four times a year.

**Cene preplate za 2016. godinu su:** 2.400 dinara za pojedince, 4.800 dinara za ustanove i 50 evra za čitače van Srbije. Preplata se može uplatiti Srpskom lekarskom društvu, ul. Džordža Vašingtona 19, 11000 Beograd, na tekući račun 205-8041-21 (Komercijalna banka AD, Beograd), sa pozivom na broj 04/1710, imenom časopisa i godinom za koju se pretplata uplaćuje. Sve dodatne informacije mogu se dobiti na telefon 011/3245-149.

**Subscriptions prices for the year 2016 are:** 2,400 RSD for individuals, 4,800 RSD for institutions, and 50 Euros for readers outside Serbia. Subscription order: Serbian Medical Society, Džordža Vašingtona 19, 11000 Belgrade; details of payment: bank account number 205-8041-21 (Komercijalna banka AD, Belgrade), invoice number 04/1710, with the name of the journal and the year for which you subscribe; beneficiary: Serbian Medical Society. For further information, please contact us on stomglas@bvcom.net.

**Finansijsku podršku izdavanju časopisa pruža**  
Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije.

**The publishing of the Journal is financially supported by** the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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

[www.stomglas.org.rs](http://www.stomglas.org.rs)



# Stomatološki glasnik Srbije

## Serbian Dental Journal

**Izдавач** 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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**Printed by**  
JP „Službeni glasnik“, Beograd

**Broj primeraka**  
**Number of copies**  
500

## Contents / Sadržaj

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<b>REČ UREDNIKA .....</b>	107
<b>ORIGINAL ARTICLES / ORIGINALNI RADOVI</b>	
Biljana Andjelski-Radičević, Maja Milošević, Ivan Dožić	
The influence of sampling method on electrolyte concentrations, pH and buffer capacity of saliva in healthy individuals .....	109
Uticaj metode uzorkovanja pljuvačke na koncentraciju elektrolita, pH vrednost i puferski kapacitet zdravih ispitanika	
Marija Obradović, Olivera Dolić, Jovan Vojinović, Slava Sukara	
Association between feeding habits and severe - early childhood caries in children up to 24 month old .....	117
Povezanost navika u ishrani sa pojmom cirkularnog karijesa u uzrastu dece do 24 meseca	
Jelena Krunić, Irena Mladenović, Nikola Stojanović	
Dental pulp pain in young and postmenopausal women: a pilot study .....	125
Pulpni bol kod žena u reproduktivnoj dobi i menopauzi: pilot studija	
<b>CASE REPORTS / PRIKAZI IZ PRAKSE</b>	
Ana Todorović, Danica Popović, Igor Djordjević, Vojkan Lazić	
Fiber reinforced composite bridge as replacement for missing upper permanent lateral incisor – a case report .....	133
Vlaknima ojačan kompozitni most kao zamena za neiznikli gornji stalni lateralni sekutić – prikaz bolesnika	
Slavoljub Tomić, Bojana Davidović	
Peripheral giant cell granuloma – case report .....	139
Periferni gigantocelularni granulom – prikaz bolesnika	
<b>DENTAL LINKS / STOMATOLOŠKI LINKOVI .....</b>	145
<b>DA LI STE PAŽLJIVO ČITALI RADOVE? .....</b>	146
<b>UPUTSTVO AUTORIMA ZA PRIPREMU RADA .....</b>	148
<b>INSTRUCTIONS FOR AUTHORS .....</b>	150



*Bojim se onih koji više mogu nego što znaju.  
Ako im se suprotstavimo, oni će nam pokazati  
da još više mogu nego što znaju.*

Dušan Radović

Ovaj citat je možda najbolji pokazatelj sadašnjeg trenutka u svim sferama života. To najbolje odslikava vreme i uslove u kojima bitiše većina ljudi na ovim prostorima. Iako je velikan pisanje i izgovorene reči ovo izgovorio još pre četiri decenije, njegova aktuelnost nimalo ne manjka ni danas.

Sve ovo naravno uključuje i brojna pitanja koja slobodoumni ljudi često postavljaju i sebi i drugima u nadi da bi odgovori na njih mogli iznedriti bolju budućnost. Zbog tih pitanja će ovaj urednički komentar biti možda malo neuobičajen.

Da li naša svakodnevica i sve što nam se dešava pokazuju da još uvek živimo u prošlosti? A činjenica je da nam je društvena stvarnost takva da su i te kako aktuelni i Radoje Domanović, i Branislav Nušić, ali i nešto „mlađi“ Dušan Radović.

Da li je naša budućnost svetla samo u kontaminiranom medijskom prostoru i ispraznoj prići o „svekolikim“ uspesima? I ima li uopšte budućnosti tamo gde je duboka provalja između društvene realnosti i aktuelnog propagiranja stvarnosti.

Može li se ići napred u društvu i sistemu gde su prave vrednosti u svim sferama skoro iskorijenjene, a kultura, umetnost i nauka marginalizovani? Mogu li o kulturi odlučivati ljudi koji „ne konzumiraju kulturu“, o nauci oni koji poistovećuju politiku sa naukom, a o umetnosti oni koji su sa njom u dubokom raskoraku.

Može li se ići napred tamo gde je sloboda mišljenja „incident“, a stvaralačka kritika „jeres“? Ima li budućnosti tamo gde neuki i nekompetentni ljudi odlučuju o svemu, pa čak i o važnim naučnim i stručnim pitanjima?

Može li naučna i stručna inferiornost iznedriti uspešnog istraživača i naučnika? A tamo gde način sticanja znanja nije ni važan ni validan, odnosno tamo gde se naučna kompetentnost stiče na osnovu nekih „posebnih“ parametara koji nemaju nikakve veze ni sa naukom ni sa strukom, budućnost je daleka i besperspektivna.

Živimo u dinamičkom, a ne statičkom svetu, pa je i životni teatar vrlo raznolik. Zato je teško uživati samo u dramama i predstavama gde je jedan glumac ujedno i režiser i scenograf i kostimograf, a često i jedini kritičar.

U naučnim krugovima kompetentnost se lako može proveriti. Stručnost je obično vidljiva, a naučna kompetentnost javna i lako dostupna, jer je to lična karta svakog istraživača. Uvek su problem samo „ljudski“ kvaliteti jer su teško proverivi.

Teško je koračati u budućnost tamo gde je propaganda važnija od istine. Ne mogu nas izvući lažne diplome i prepisani doktorati, već samo znanje, poštjenje i istina.

Ovaj urednički komentar će završiti onako kako sam i počeо i opet citatom velikog i uvek aktuelnog Dušana Radovića: „Neki nesposobni ljudi dogurali su daleko. Ko je nesposoban može raditi sve, a sposobni samo ono što umeju.“

*Prof. dr Slavoljub Živković*



# The influence of sampling method on electrolyte concentrations, pH and buffer capacity of saliva in healthy individuals

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## SUMMARY

**Introduction** Saliva is a complex secretion, which plays an important role in maintenance of oral health. Analysis of saliva is fast, simple and non-invasive, and it is increasingly used as a biological sample for determination of various biochemical markers. The aim was to determine the influence of unstimulated saliva collection methods for measuring electrolytes concentration (sodium, potassium, calcium), pH and buffer capacity of saliva in healthy subjects.

**Material and methods** 30 healthy subjects, males and females, aged 18 to 20 years, without oral and systemic diseases were included in the study. Unstimulated saliva samples were taken using a special tube (Salivette) and via direct spitting into the test tube. The concentrations of sodium and potassium were determined by flame emission photometry while spectrophotometry was used for calcium concentration. For the analysis of pH value of saliva pH-meter was used, while saliva buffer capacity was determined by titration with HCl (0.005 mol/L).

**Results** The level of sodium in unstimulated saliva collected in test tubes was  $8.43 \pm 3.92$  mmol/L and in special tubes  $7.90 \pm 4.33$  mmol/L. Potassium level in unstimulated saliva collected in test tubes was  $13.62 \pm 0.99$  mmol/L while in special tubes it was  $13.54 \pm 0.94$  mmol/L. Mean values of sodium and potassium in unstimulated saliva didn't show statistically significant difference in their concentrations between the two methods of collecting saliva. In contrast to these electrolytes, calcium concentration was higher in the samples of saliva collected with special tubes ( $2.04 \pm 1.05$  mmol/L) compared to the samples taken by direct spitting into the test tube ( $1.38 \pm 1.18$  mmol/L) with statistically significant difference ( $p < 0.05$ ). By analyzing the pH of unstimulated saliva it was found that the average pH value of saliva collected with special tubes was  $7.05 \pm 0.32$ , and after direct spitting into test tubes it was  $7.35 \pm 0.41$ . Buffer capacity of saliva in healthy subjects was lower after taking with special tubes ( $5.18 \pm 0.74$ ) compared to test tubes ( $5.36 \pm 0.85$ ), but without statistical difference.

**Conclusion** Unstimulated saliva collecting methods using cotton pads (salivette) and direct spitting in the test tube did not affect the value of pH, buffer capacity, the concentrations of sodium and potassium, but affected the concentration of calcium in saliva from healthy subjects.

**Keywords:** saliva; electrolytes; pH; buffer capacity; salivette

## INTRODUCTION

Saliva is body fluid of complex composition with main role of continuous wetting and washing oral mucosa and teeth. Ultrafiltration of blood in the acinar cells of the salivary glands produce primary saliva, which biochemical composition changes passing through the duct system, so final saliva is hypotonic in relation to blood plasma [1]. Mixing secretions from the three pairs of large salivary glands (parotid, sublingual, submandibular), small salivary glands and gingival fluid forms the total (mixed) saliva in the mouth. Quantity and composition of extracted saliva is affected by the time of day, degree of hydration, body position, mental stimulation, medications, habits (eg. cigarette smoking), general health, oral diseases and others [2].

Saliva components are coming from salivary glands, blood or gingival fluid. Water is about 99%, and the rest are organic molecules (proteins, glycoproteins, lipids), electrolytes, desquamated epithelial cells, nutritious particles, microorganisms... [3]. Saliva has numerous roles and an important function in the maintenance of oral

homeostasis, ie. permanent composition of oral environment. This is primarily related to the self-cleaning of oral cavity (water, amylase), chewing, swallowing, speaking, maintaining the stability of prosthetic restorations in the oral cavity, antimicrobial protection (proteins and glycoproteins), antioxidant role (uric acid, bilirubin, glutathione), buffer role (phosphates and bicarbonates).

Saliva contains a variety of electrolytes: bicarbonate, calcium, chloride, fluoride, iodide, magnesium, phosphate, sodium, potassium, sulphate, thiocyanate, etc [4]. However, there are significant differences between the electrolyte concentrations in saliva and blood plasma [5]. There are also differences in electrolyte concentrations between stimulated and unstimulated saliva. Increased secretion of saliva (acidic food) increases the concentration of sodium, chloride, bicarbonate, and reduces the concentration of potassium and phosphate compared to unstimulated saliva [6].

To maintain the acid-base balance in saliva the most important are bicarbonate, phosphate and protein buffers. They maintain the pH value of saliva within the normal



**Figure 1.** Saliva sampling using special tubes (Salivette®)  
**Slika 1.** Uzimanje pljuvačke pomoću salivete

range from 6.1 to 7.8 [7]. Buffer capacity (BC) of saliva depends on the flow of saliva, therefore phosphate buffer is primary buffer in unstimulated saliva, which gives it slightly acidic character (pH about 6.1), while the main buffer in stimulated saliva is bicarbonate buffer, which makes it slightly alkaline (pH about 7.8).

In the recent years, saliva has been increasingly used as biological material that could be collected in a simple, painless and safe way. Saliva sampling procedure is of great importance for experimental and clinical researches, and the establishment of precise diagnostic protocols [8]. In the number of studies biomolecules in saliva were analyzed in oral and systemic diseases, as well as concentrations of certain medicaments and psychotropic substances [9, 10]. Unstimulated saliva is tested more often than stimulated, because materials that stimulate saliva secretion can change its biochemical composition. Method of sampling and storage of saliva before analysis mainly affects the findings of biochemical markers [3]. Some compounds in saliva have short half-life and the sample must be analyzed as soon as possible, while other saliva compounds are stable for a long time [11].

The aim of this study was to determine the influence of saliva collection methods in the measurement of electrolytes concentration (sodium, potassium, calcium), pH and buffer capacity of unstimulated saliva in healthy subjects.

## MATERIAL AND METHODS

The study included 30 healthy subjects, 16 male and 14 female, aged between 18 and 20 years. The main criteria for selection were young subjects with no oral and systemic diseases. Unstimulated saliva was collected in the morning, between 9 am and 11 am. Individuals were instructed not to drink, eat, smoke, chew chewing gum or brush their teeth for at least 30 minutes before examination. Saliva sampling was done in two ways. Subjects were comfortably seated and after few minutes of relaxation, they were trained to avoid swallowing saliva and asked to

lean forward and spit all saliva they passively produced into a plastic test tube. After 5 minutes of rest, saliva was passively flowed into test tubes. After a short break, saliva was sampled using special tubes Salivette®, Sarstedt, Germany (Figure 1), by placing a cotton pad on the floor of the mouth, for 3 to 5 minutes. All samples were centrifuged at 3000 rpm for 10 minutes.

pH value of saliva was measured immediately after sampling using pH meter (Martini Instruments, USA). As per modified Ericsson's method [12], buffer capacity of saliva was determined by adding 0.5 mL HCl (0.005 mol/L) in 0.5 mL of each saliva sample [13]. That way pH value was disturbed in order to determine the function of salivary buffer to maintain the pH in physiological range. The solution was mixed using magnetic stirrer and incubated at the room temperature for 30 sec, and then the pH was measured using pH-meter.

Sodium and potassium levels in saliva were measured using flame photometry (Hospitex Diagnostics, Italy) and calcium was measured using spectrophotometric method (RT-1904C, USA). All tests were performed in the Laboratory for Biochemistry and Haematology, Faculty of Dental Medicine in Belgrade.

Data were analyzed using Statistical Package for Social Sciences (SPSS Inc.). Statistical comparisons were performed using Student's t-test and  $p < 0.05$  was considered statistically significant.

## RESULTS

Mean concentrations of sodium ( $7.90 \pm 4.33$  mmol/L) and potassium ( $13.54 \pm 0.94$  mmol/L) in unstimulated saliva were slightly lower after sampling saliva with special tubes compared to passive spitting into the test tubes, but without statistically significant differences (Table 1). As opposed to these electrolytes comparison, the concentration of calcium in saliva samples taken with special tubes was higher ( $2.04 \pm 1.05$  mmol/L) than by passive spitting into the test tubes ( $1.38 \pm 1.18$  mmol/L). It was

**Table 1.** Biochemical analysis of saliva in healthy subjects**Tabela 1.** Rezultati biohemijske analize pljuvačke zdravih ispitanika

Marker	Sampling method Metoda uzorkovanja	Mean value $\pm$ SD Srednja vrednost $\pm$ SD	p value p vrednost
<b>Sodium</b> (mmol/L) <b>Natrijum</b>	Test tube Epruveta	8.43 $\pm$ 3.92	p = 0.512
	Special tube Saliveta	7.90 $\pm$ 4.33	
<b>Potassium</b> (mmol/L) <b>Kalijum</b>	Test tube Epruveta	13.62 $\pm$ 0.99	p = 0.414
	Special tube Saliveta	13.54 $\pm$ 0.94	
<b>Calcium</b> (mmol/L) <b>Kalcijum</b>	Test tube Epruveta	1.38 $\pm$ 1.18	p = 0.026*
	Special tube Saliveta	2.04 $\pm$ 1.05	
<b>pH value</b> <b>pH vrednost</b>	Test tube Epruveta	7.35 $\pm$ 0.41	p = 0.102
	Special tube Saliveta	7.05 $\pm$ 0.32	
<b>Buffer capacity (BC)</b> <b>Puferski kapacitet (PK)</b>	Test tube Epruveta	5.36 $\pm$ 0.85	p = 0.284
	Special tube Saliveta	5.18 $\pm$ 0.74	

SD – standard deviation; \* – statistical significance  
 SD – standardna devijacija; \* – statistička značajnost

found that there was statistically significant difference in calcium concentrations in unstimulated saliva between the two different methods ( $p < 0.05$ ) (Table 1).

Mean pH value of unstimulated saliva from healthy subjects, sampled by passive spitting into the test tubes was 7.35 whereas it was 7.05 when saliva was sampled using special tubes but this difference was not statistically significant (Table 1).

Buffer capacity of saliva is important to maintain the pH value in the oral cavity and for teeth remineralisation. The mean value of buffer capacity in the samples obtained via spitting into the test tubes was  $5.36 \pm 0.85$ , slightly higher than the average value of the buffer capacity  $5.18 \pm 0.74$  obtained via salivette (Table 1). Individual buffer capacity was ranked in one of the three categories: high BC (pH higher than 5.5), medium BC (pH 4.5 to 5.5), low BC (pH less than 4.5) [13]. From the total of 30 samples, high saliva buffer capacity sampled via passive spitting collection methodology was found in 14 patients (46.7 %), as opposed to saliva collected with special tubes, where most of the samples had medium BC (15 subjects

50 %) (Figure 2). Student's t-test showed no significant difference between the measured values of buffer capacity depending on the sampling method.

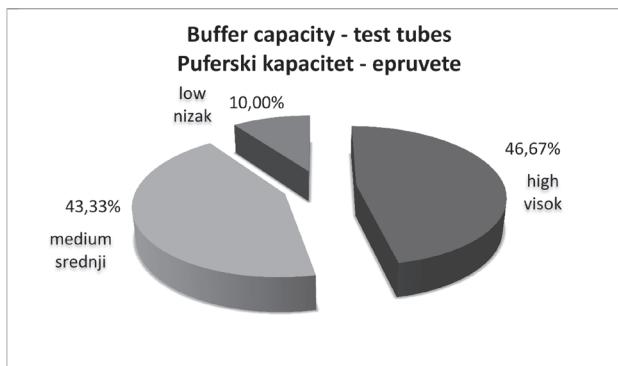
## DISCUSSION

Saliva is biological fluid important for maintaining oral health. Salivary biomarkers can be significant indicators of some oral and systemic diseases [9, 14-16]. Currently there are standardized methods for routine determination of some markers in saliva such as narcotics [17], steroid hormones [18-20], peptides and various medicaments [21-23].

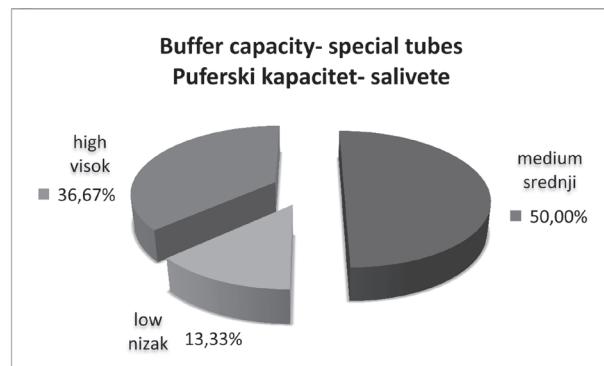
For biochemical markers determination in saliva, standardized procedures of sampling, storage and preparation of saliva are important. Currently, there are no universally accepted techniques for saliva sampling, even though they can affect reliability of results [24, 25]. Data from literature suggest that the method of collecting saliva from healthy subjects affects the concentration of C-reactive protein, immunoglobulin E, myoglobin [26], alpha-amylase [27], lactoferrin [28].

In the current study, the aim was to determine the validity of saliva sampling methods during the preanalytical phase. The results showed no statistically significant difference in the concentrations of sodium and potassium in saliva collected by passive salivating into test tubes or by collection into special tubes. Mean concentrations of tested electrolytes in unstimulated mixed saliva did not differ from results obtained in other studies [29, 30]. Sodium ion from saliva is important in maintaining osmotic pressure of the extracellular fluid. Studies have shown that in salivary glands' diseases (Sjögren's syndrome), due to absorption disorders at the level of secretory tubules of epithelial cells, sodium concentration in saliva is elevated compared to healthy subjects [31]. Unlike sodium, potassium is the principal cation in the intracellular liquid. However, due to exchange of sodium and potassium at the level of the salivary glands' secretory duct, potassium concentration is increased in unstimulated saliva in relation to blood plasma.

Saliva is saturated with calcium ions that are in equilibrium with the same ions of hydroxyapatite in tooth enamel. In saliva calcium is mainly in ionic form (about 50 %), and the rest is in the complex with organic ions



**Figure 2.** Buffer capacity of saliva samples in healthy subjects  
**Slika 2.** Puferski kapacitet u uzorcima pljuvačke zdravih ispitanika



(citrates) and salivary proteins (statherin, histatine, proline-rich glycoproteins) [32]. The concentration of calcium in saliva varies depending on the protein concentration, saliva flow rate and secretion of salivary glands [33]. Some authors have pointed out that calcium concentration in saliva significantly increases with aging [34]. Other authors think that calcium is unstable, because it can precipitate, or forms complexes with proteins, phosphates, citrate and lactate, so recommendation is to do analysis immediately after collecting saliva samples [35]. The current study showed statistically significant difference in calcium concentrations in unstimulated saliva between the two saliva-sampling methods. It was found that calcium concentration in saliva samples from healthy subjects was lower when samples were collected into the test tubes by passive spitting (1.38 mmol/L) compared to sample collection in special tubes (2.04 mmol/L). This could be explained by the fact that cotton pads absorb proteins and other molecules inside the cartridge during saliva sampling, or during centrifugation they attach to the cotton fibres preventing the formation of calcium complex.

There is insufficient data in the literature on the impact of saliva sampling method to pH change. The results of the current study showed no statistically significant difference between the two methods of collecting saliva. Mean salivary pH value was about 7 and it was in accordance with the results of other authors [36]. Some researches have shown that pH value and flow rate of saliva depend on the degree of body hydration, exposure to light stimuli and sensations, as well as body position [37]. Authors have noted that body dehydration of 8% can reduce the flow of saliva up to 100%, which has an impact on other biochemical markers. Recent research [38] found that females had lower pH compared to men in younger population. Some researchers have demonstrated statistically significant increased pH values in smokers compared to non-smokers [39], and others have pointed to the lowering of pH values with a consequent increase of calcium concentration in saliva [40, 41].

Salivary buffer system has an important role in maintaining pH value of the oral environment within the normal range (6.1 to 7.8), remineralization of teeth and prevention of dental caries. It depends on the concentration of bicarbonate [42] and has been correlated to the flow of saliva. In the current study no statistically significant difference was found between the buffer capacities of saliva collected in the special tubes, relative to the test tubes. Collection of saliva in salivette with cotton pad can absorb substances that are of importance for salivary buffer capacity.

## CONCLUSION

Unstimulated saliva collecting methods using cotton pads (salivette) and via direct spitting into the test tube did not affect pH value, buffer capacity and concentrations of sodium and potassium in saliva. However, in saliva calcium level determining, more precise results were obtained by taking samples directly via spitting into the test tube compared to the method of collecting saliva in special tubes (cotton pads).

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Received: 01.03.2016 • Accepted: 07.06.2016

# Uticaj metode uzorkovanja pljuvačke na koncentraciju elektrolita, pH vrednost i puferski kapacitet zdravih ispitanika

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## KRATAK SADRŽAJ

**Uvod** Pljuvačka je složen sekret koji ima značajnu ulogu u održavanju oralnog zdravlja. Analiza pljuvačke je brza, jednostavna i neinvazivna, pa se sve češće koristi kao biološki uzorak za određivanje različitih biohemijskih markera. Cilj ovog rada bio je da se proceni uticaj metode sakupljanja nestimulisane pljuvačke na koncentraciju elektrolita (natrijuma, kalijuma, kalcijuma), pH i puferski kapacitet pljuvačke kod zdravih ispitanika.

**Materijal i metode rada** U istraživanje je uključeno 30 zdravih ispitanika muškog i ženskog pola, starosti od 18 do 20 godina, bez oralnih i sistemskih oboljenja. Uzorci nestimulisane pljuvačke su uzimani pomoću specijalnih epruveta – saliveta, i direktnim ispljuvavanjem u epruvete. Koncentracije natrijuma i kalijuma u pljuvački određivane su metodom plamene emisione fotometrije, a koncentracija kalcijuma metodom spektrofotometrije. Za analizu pH pljuvačke korišćen je pH-metar, a puferski kapacitet pljuvačke je određivan titracijom sa HCl (0,005 mol/L).

**Rezultati** Koncentracija natrijuma u nestimulisanoj pljuvački sakupljenoj epruvetama iznosila je  $8,43 \pm 3,92$  mmol/L, a u salivetama  $7,90 \pm 4,33$  mmol/L. Koncentracija kalijuma u nestimulisanoj pljuvački sakupljenoj epruvetama iznosila je  $13,62 \pm 0,99$  mmol/L, a u salivetama  $13,54 \pm 0,94$  mmol/L. Analiza natrijuma i kalijuma u nestimulisanoj pljuvački nije pokazala statistički značajnu razliku u njihovoj koncentraciji između dve metode sakupljanja pljuvačke. Za razliku od ovih elektrolita, koncentracija kalcijuma je bila veća u uzorku pljuvačke sakupljene salivetama ( $2,04 \pm 1,05$  mmol/L) u odnosu na uzorak koji je uzet direktnim ispljuvavanjem u epruvetu ( $1,38 \pm 1,18$  mmol/L), sa statistički značajnom razlikom ( $p < 0,05$ ). Analizom pH nestimulisane pljuvačke utvrđeno je da je srednja pH vrednost pljuvačke sakupljene salivetama  $7,05 \pm 0,32$ , a direktnim ispljuvavanjem u epruvete  $7,35 \pm 0,41$ . Puferski kapacitet pljuvačke zdravih ispitanika je bio niži nakon uzimanja salivetama ( $5,18 \pm 0,74$ ) nego epruvetama ( $5,36 \pm 0,85$ ), ali bez statistički značajne razlike.

**Zaključak** Metode sakupljanja nestimulisane mešovite pljuvačke pomoću pamučnih uložaka (salivete) i direktnim ispljuvavanjem u epruvete ne utiču na vrednost pH, puferski kapacitet, koncentraciju natrijuma i kalijuma u pljuvački, ali utiču na koncentraciju kalcijuma u pljuvački zdravih ispitanika.

**Ključne reči:** pljuvačka; elektroliti; pH; puferski kapacitet; salivete

## UVOD

Pljuvačka (saliva) je složen sekret u usnoj duplji koji neprekidno vlaži i spira oralnu sluzokožu i zube. Ultrafiltracijom krvi u acinusnim ćelijama pljuvačnih žlezda nastaje primarna pljuvačka, čiji se biohemijski sastav menja prolaskom kroz sistem izvodnih kanalića, tako da je definitivna pljuvačka hipotonična u odnosu na krvnu plazmu [1]. Mešanjem sekreta iz tri para velikih pljuvačnih žlezda (parotidne, podjezične, podvilične), malih pljuvačnih žlezda, gingivalne tečnosti formira se ukupna (mešovita) pljuvačka u usnoj duplji. Na količinu i sastav izlučene pljuvačke utiče doba dana, stepen hidratacije organizma, položaj tela, psihička stimulacija, lekovi, navike (npr. pušenje cigareta), opšte zdravstveno stanje, oboljenja usne duplje i dr. [2].

Sastojci pljuvačke potiču iz pljuvačnih žlezda, krvi ili gingivalne tečnosti. Vode ima oko 99%, a ostatak su organski molekuli (proteini, glikoproteini, lipidi), elektroliti, deskvamirane epitelne ćelije, hranljive čestice, mikroorganizmi... [3]. Uloge sastojaka pljuvačke su brojne i imaju značajnu funkciju u održavanju oralne homeostaze, tj. stalnog sastava oralne sredine. To se pre svega odnosi na samočišćenje usne duplje (voda, amilaza), žvakanje, gutanje, govor, održavanje stabilnosti protetskih nadoknada u usnoj duplji, antimikrobnu zaštitu (proteini i glikoproteini), antioksidativnu ulogu (mokraćna kiselina, bilirubin, glutation), pufersku ulogu (fosfati i bikarbonati).

U pljuvački su prisutni mnogi elektroliti: bikarbonati, kalcijum, hloridi, fluoridi, jodidi, magnezijum, fosfati, natrijum, kalijum, sulfati, tiocijanati i dr. [4]. Međutim, postoje značajne razlike u koncentraciji elektrolita u pljuvački u odnosu na krvnu

plazmu [5]. Takođe su ustanovljene razlike u koncentraciji elektrolita između stimulisane i nestimulisane pljuvačke. Povećanim lučenjem pljuvačke (kisela hrana) povećava se koncentracija natrijuma, hlorida, bikarbonata, a smanjuje koncentracija kalijuma i fosfata u odnosu na nestimulisanoj pljuvačku [6].

Za održavanje acidobazne ravnoteže u pljuvački najznačajniji su bikarbonatni, fosfatni i proteinski puferi. Oni održavaju pH vrednost pljuvačke u fiziološkim granicama, od 6,1 do 7,8 [7]. Puferski kapacitet (PK) pljuvačke zavisi od protoka pljuvačke tako da je fosfatni pufer primarni pufer nestimulisane pljuvačke, koji joj daje blago kiseli karakter (pH oko 6,1), dok je bikarbonatni pufer glavni pufer stimulisane pljuvačke, koji doprinosi njenoj blagoj alkalizaciji (pH oko 7,8).

Poslednjih godina pljuvačka se sve više koristi kao biološki materijal, koji se može prikupljati na jednostavan, bezbolan i siguran način. Procedura uzorkovanja pljuvačke od velike je važnosti za eksperimentalna i klinička istraživanja, kao i za uspostavljanje preciznih dijagnostičkih protokola [8]. U brojnim studijama su analizirani biomolekuli u pljuvački kod oralnih i sistemskih oboljenja, kao i koncentracije pojedinih medicamenata i psihohemikalnih supstanci [9, 10]. Češće se ispituje nestimulisana pljuvačka, u odnosu na stimulisanoj, jer materijali koji stimulišu lučenje pljuvačke mogu dovesti do promene njenog biohemijskog sastava. Način uzorkovanja i skladištenja pljuvačke pre analize uglavnom utiče na rezultate određivanja biohemijskih markera [3]. Neka jedinjenja u pljuvački imaju kratak poluživot i uzorak se mora analizirati u najkraćem roku, dok su druga jedinjenja stabilna u pljuvački duže vreme [11].

Cilj ovog istraživanja bio je da se proveri uticaj metode sakupljanja nestimulisane pljuvačke na koncentraciju elektrolita (natrijuma, kalijuma, kalcijuma), pH i puferski kapacitet pljuvačke kod zdravih ispitanika.

## MATERIJAL I METODE

U istraživanje je uključeno 30 zdravih ispitanika, 16 muškaraca i 14 žena, starosti između 18 i 20 godina. Osnovni kriterijum za uključivanje u studiju su bili ispitanici mlade populacije bez oralnih i sistemskih oboljenja.

Nestimulisana pljuvačka je sakupljana u prepodnevnim satima, između 9 i 11 h. Ispitanici su se pridržavali protokola uzimanja pljuvačke i to da 30 minuta pre sakupljanja pljuvačke ne Peru zube, ne konzumiraju hranu, vodu, duvan i gumi za žvakanje. Uzorkovanje pljuvačke je urađeno na dva načina. Ispitanici su tokom sakupljanja pljuvačke bili u sedećem položaju, sa glavom nagnutom prema napred. Nakon pet minuta mirovanja, pljuvačka je pasivnim slijanjem sakupljana u plastične epruvete. Posle kratkog odmora, pljuvačka je uzorkovana i pomoću specijalnih epruveta Salivette®, Sarstedt, Nemačka (Slika 1), postavljanjem pamučnog uloška na pod usne duplje, u trajanju od 3 do 5 minuta. Uzorci nestimulisane pljuvačke sakupljeni pasivnim ispljuvavanjem i pomoću saliveta centrifugovani su na 3000 obrt./min. u trajanju od 10 minuta.

Neposredno nakon uzimanja uzorka pljuvačke urađeno je merenje pH vrednosti na pH-metru (Martini Instruments, USA). Modifikovanom metodom po Ericssonu [12] određen je puferski kapacitet pljuvačke tako što je u 0,5 mL svakog uzorka pljuvačke dodato po 0,5 mL HCl (0,005 mol/L) [13]. Time se remeti pH vrednost kako bi se utvrdila funkcija pufera pljuvačke da održe pH u fiziološkim granicama. Rastvor je promešan magnetnom mešalicom i inkubiran na sobnoj temperaturi 30 s, a zatim pehametrom meren pH.

Koncentracije natrijuma i kalijuma u pljuvački merene su na plamenom fotometru (Hospitex Diagnostics, Italija), a koncentracija kalcijuma određena je metodom spektrofotometrije (RT-1904C, USA). Sve analize su urađene u Laboratoriji za biohemiju i hematologiju Stomatološkog fakulteta Univerziteta u Beogradu.

Za analizu rezultata korišćen je Studentov t-test. Statistička značajnost je određena na nivou  $p < 0,05$ . Materijal za studiju obrađen je kompjuterski u programima SPSS v.13.0 (SPSS Inc.) i Microsoft Office 2003.

## REZULTATI

Srednje vrednosti koncentracije natrijuma ( $7,90 \pm 4,33$  mmol/L) i kalijuma ( $13,54 \pm 0,94$  mmol/L) u nestimulisanoj pljuvački su bile nešto niže nakon uzorkovanja pljuvačke salivetama u odnosu na pasivno ispljuvavanje u epruvete, ali bez statistički značajne razlike (Tabela 1). Za razliku od ovih elektrolita, koncentracija kalcijuma u uzorcima pljuvačke uzetim salivetama bila je veća ( $2,04 \pm 1,05$  mmol/L) u odnosu na pasivno ispljuvavanje u epruvete ( $1,38 \pm 1,18$  mmol/L). Utvrđeno je da postoji statistički značajna razlika u koncentraciji kalcijuma u nestimulisanoj pljuvački između korišćenih metoda ( $p < 0,05$ ).

Srednja pH vrednost nestimulisane pljuvačke kod zdravih ispitanika, uzorkovane pasivnim ispljuvavanjem u epruvete

iznosila je 7,35, a kod uzorkovanja salivetama 7,05 (Tabela 1) i ova razlika nije bila statistički značajna.

Puferski kapacitet pljuvačke je značajan za održavanje pH u usnoj duplji i za remineralizaciju zuba. Srednja vrednost puferskog kapaciteta u uzorcima sakupljenim metodom ispljuvavanja u epruvete iznosila je  $5,36 \pm 0,85$  i bila je nešto veća u odnosu na srednju vrednost puferskog kapaciteta  $5,18 \pm 0,74$  u uzorcima pljuvačke sakupljenim u salivete (Tabela 1). Individualni puferski kapacitet bio je rangiran u jednu od tri kategorije: visok PK (pH veći od 5,5), srednji PK (pH od 4,5 do 5,5), nizak PK (pH manji od 4,5) [13]. Od ukupno 30 uzoraka, visok puferski kapacitet pljuvačke, uzorkovane pasivnim pljuvanjem u epruvete, bio je kod 14 ispitanika (46,7%), za razliku od pljuvačke sakupljane salivetama, gde je najviše uzorka bilo sa srednjim puferskim kapacitetom kod 15 ispitanika (50%) (Slika 2). Studentovim t-testom nije ustanovljena statistički značajna razlika između izmerenih vrednosti puferskog kapaciteta u zavisnosti od metode uzorkovanja.

## DISKUSIJA

Pljuvačka je biološka tečnost veoma korisna za održavanje oralnog zdravlja. U pljuvački se mogu analizirati biomarkeri koji su značajni indikatori oralnih, ali i sistemskih oboljenja [9, 14, 15, 16]. U svetu postoje standardizovane metode za rutinsko određivanje nekih markera u pljuvački kao što su opojna sredstva (droge) [17], steroidni hormoni [18, 19, 20], peptidi, lekovi [21, 22, 23].

Tokom određivanja biohemijskih markera u pljuvački, pored standardizacije postupaka, od izuzetne važnosti je i kontrola preanalitičke faze i to uzorkovanje, čuvanje i priprema pljuvačke. Trenutno ne postoje univerzalno prihvачene tehnike za uzorkovanje pljuvačke, pa ova činjenica može da utiče na pozdanost dobijenih rezultata [24, 25]. Podaci iz literature ukazuju da metoda prikupljanja pljuvačke kod zdravih ispitanika utiče na koncentraciju C-reaktivnog proteina, imunoglobulina E, mikroglobulina [26], alfa-amilaze [27], laktoperferina [28].

U ovom istraživanju postavljen je cilj da se utvrdi valjanost metode uzorkovanja pljuvačke tokom preanalitičke faze. Rezultati pokazuju da nema statistički značajne razlike u koncentraciji natrijuma i kalijuma u pljuvački sakupljanoj pasivnim ispljuvavanjem u epruvete ili sakupljanjem u salivetama. Srednje vrednosti koncentracija ispitivanih elektrolita u nestimulisanoj mešovitoj pljuvački ne odstupaju od rezultata dobijenih u drugim istraživanjima [29, 30]. Jon natrijuma iz pljuvačke je važan u održavanju osmotskog pritiska u ekstračelijskoj tečnosti. Studije su pokazale da kod oboljenja pljuvačnih žlezda (Sjögren sindrom), zbog poremećaja apsorpcije na nivou epitelnih ćelija izvodnih kanaliča, koncentracija natrijuma u pljuvački je povišena u odnosu na zdrave ispitanike [31]. Za razliku od natrijuma, kalijum je glavni katjon u intracelijskoj tečnosti. Međutim, zbog izmene natrijuma i kalijuma na nivou izvodnih kanala pljuvačnih žlezda, koncentracija kalijuma je veća u nestimulisanoj pljuvački u odnosu na krvnu plazmu.

Pljuvačka je zasićena jonom kalcijuma, koji se nalaze u ravnotežnom odnosu sa istim jonom iz hidroksiapatita zubne gleđi. U pljuvački se kalcijum nalazi uglavnom u jonskom obliku (oko 50%), a ostatak u kompleksu sa organskim jonom (citrati) i proteinima pljuvačke (staterin, histatin, prolinom-

bogati glikoproteini) [32]. Koncentracija kalcijuma u pljuvački varira u zavisnosti od koncentracije proteina, protoka pljuvačke i sekrecije pljuvačnih žlezda [33], a neki autori su ukazali da se sa starenjem značajno povećava koncentracija kalcijuma u pljuvački [34]. Drugi autori smatraju da je kalcijum nestabilan, jer može da precipitira ili gradi komplekse sa proteinima, fosfatima, citratima i laktatima i preporučuju analiziranje odmah posle sakupljanja uzoraka pljuvačke [35]. Ova studija je pokazala da postoji statistički značajna razlika u koncentraciji kalcijuma u nestimulisanoj pljuvački između dve korišćene metode sakupljanja pljuvačke. Utvrđeno je da je koncentracija kalcijuma kod zdravih ispitanika niža u uzorcima pljuvačke koji su uzimani pasivnim ispljuvavanjem u epruvete ( $1,38 \text{ mmol/L}$ ) u odnosu na sakupljanje uzoraka salivetama ( $2,04 \text{ mmol/L}$ ). Ovo bi se moglo objasniti činjenicom da je tokom uzimanja pljuvačke pamučnim ulošcima saliveta moguće da se proteini i drugi molekuli apsorbuju unutar uloška, ili da se centrifugovanjem još više vezuju za pamučna vlakna, čime je sprečeno kompleksiranje kalcijuma.

U literaturi nema dovoljno podataka o uticaju načina uzorkovanja pljuvačke na promene vrednosti pH. Dobijeni rezultati pokazuju da ne postoji statistički značajna razlika između dve metode sakupljanja pljuvačke. Srednja pH vrednost pljuvačke je bila oko 7 i u saglasnosti je sa rezultatima drugih autora [36]. U nekim istraživanjima je dokazano da pH vrednost i protok pljuvačke zavise od stepena hidratacije organizma, izloženosti stimulansima i svetlosnim senzacijama, kao i od položaja tela [37]. Autori navode da dehidratacija tela od 8% može redukovati protok pljuvačke i do 100%, što ima uticaj i na druge biohe-

mjske markere. U novijim istraživanjima [38], kod ispitanika mlađe populacije utvrđena je niža pH vrednost pljuvačke kod ženskih ispitanika u odnosu na muške. Neki istraživači su dokazali statistički značajno povećanje pH vrednosti kod pušača u odnosu na nepušače [39], a drugi su ukazali na smanjenje pH vrednosti sa posledičnim povećanjem koncentracije kalcijuma u pljuvački [40, 41].

Puferski sistem pljuvačke je značajan za održavanje pH vrednosti oralne sredine u fiziološkim granicama (6,1–7,8), uticaj na remineralizaciju zuba i prevenciju zubnog karijesa. Zavisan je od koncentracije bikarbonata [42] i u korelaciji je sa protokom pljuvačke. U ovoj studiji pokazano je da nema statistički značajne razlike između puferskog kapaciteta pljuvačke sakupljane u salivetama i epruvetama. Sakupljanje pljuvačke u salivete sa pamučnim uloškom može da apsorbuje supstance koje su od značaja za puferski kapacitet pljuvačke.

## ZAKLJUČAK

Metode sakupljanja nestimulisane mešovite pljuvačke pomoći pamučnih uložaka (salivete) i direktnim ispljuvavanjem u epruvete ne utiču na vrednost pH, puferski kapacitet, koncentraciju natrijuma i kalijuma u pljuvački. Međutim, kod određivanja koncentracije kalcijuma u pljuvački, uzimanjem uzoraka direktnim ispljuvavanjem u epruvetu dobijaju se precizniji rezultati u odnosu na metodu sakupljanja pljuvačke salivetama (pamučni ulošci).

# Association between feeding habits and severe - early childhood caries in children up to 24 month old

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## SUMMARY

**Introduction** During the first two years of life children's nutrition is mostly based on frequent, liquid and sweetened meals which can cause Severe-Early Childhood Caries (S-ECC) development. The aim of this research was to determine the relationship between dietary habits and S-ECC in children up to 24 month-old living in Banja Luka, Bosnia and Herzegovina.

**Methods** Cross-sectional study included representative sample of 192 children. Before dental examination of children, each parent/caregiver was interviewed about the basic info, socio-demographic characteristics and children's eating habits. The questionnaire was conducted as interview ("face to face"). Subjects were divided into two groups: the first group - children with S-ECC and the second group - caries free children. For statistical analysis and presentation of results SPSS 16.0 for Windows, MS Office Word and Microsoft Office Excel were used.

**Results** In the study sample 34.9% of children were suffering from S-ECC. About 50% of children who were breast-fed at night after first tooth eruption had S-ECC. The use of baby bottle with milk or other sweetened content during bedtime and during the night was identified as significant caries risk factor ( $P < 0.05$ ).

**Conclusion** Nighttime breastfeeding, use of bottle with milk during bedtime/nighttime or other sweetened content during night after eruption of first primary tooth were strongly associated with S-ECC in the examined children.

**Keywords:** breastfeeding; Severe - Early Childhood Caries; nighttime breastfeeding; baby bottle; risk factors

## INTRODUCTION

Early childhood is crucial for proper development and maintaining oral health. Early childhood caries (ECC) is one of major public health problems in the world [1-7]. According to the research it is one of the most common chronic infectious disease in early childhood that is due to its multifactorial etiology difficult to control [8, 9].

Aggressive form of the disease - Severe Early Childhood Caries (S-ECC) can occur as soon as the deciduous tooth appears in the mouth, with localization on caries immune sites or smooth surfaces of the tooth [10]. The disease mostly begins as an off-white chalky change on the vestibular surface of the enamel along the gum line and sometimes on oral surface of primary maxillary incisors. Caries destruction of the tooth can be quite rapid and soon it can spread on other teeth. If child does not receive an appropriate treatment the disease can lead to pulp involvement, pain and other complications.

Similar to other forms of tooth decay S-ECC etiology includes interaction of three main factors: cariogenic microorganisms, fermentable carbohydrates and a host (tooth) during period of time [11]. Besides, specificities of an early age, like immature immune system, newborn teeth in the stage of post eruptive mineralization, hy-

poplastic changes, a special type of food (mostly liquid based) are considered to be risk factors for S-ECC development [12, 13]. In addition to primary risk factors, there is a wide range of secondary factors that can indirectly contribute to development of carious lesions in primary teeth, but they are still under investigation [8-10].

Breastfeeding is natural, desirable type of feeding of a newborn and undoubtedly has great impact on overall health and development. Human milk is specific and its composition ideally suits human babies, opposite to substitutions of different origin (adapted infant formula, animal milk) that differs greatly in content. Breastfeeding is of great significance for proper growth, it provides optimal nutritional ingredients, immune protection to infants, and minimizes economic impact on families. The World Health Organization recommends breastfeeding until 24 months of age [14]. However, the American Academy of Pediatric Dentistry (AAPD) recommends that breastfeeding should stop around 12 months of age, even before, as soon as the first primary tooth erupts (around six months of age), because they consider that a longer duration of this habit contribute to development of an aggressive form of Early Childhood Caries (S-ECC) [10].

Children's diet in the first two years of life is mostly based on frequent, liquid and often sweetened meals.

A special role in the etiology of dental caries in early childhood plays an inappropriate use of baby bottle especially during bedtime and nighttime. When a child falls asleep, liquid from a bottle is poured onto maxillary incisors, representing an outstanding basis for the development of cariogenic bacteria, especially because during the night the secretion of saliva is reduced [15].

In Banja Luka, Republic of Srpska, there is no organized prevention program in dentistry. There is no systematic oral health promotion and education of parents, there is lack of data regarding dental pathology and related risk factors in an early age because the practice of the first dental visit around the first birthday is not yet established. Unfortunately, the first dental examination is required only when enrolling into primary school.

The aim of this study was to determine relationship between dietary habits and development of Severe-Early Childhood Caries (S-ECC) in children up to 24-month-old living in Banja Luka, Bosnia and Herzegovina.

## MATERIAL AND METHODS

The survey was conducted among children up to 24 month old, between July 2012 and July 2013. The cross-sectional retrospective study included 192 children of both genders that represented 10% sample (even slightly larger) of a total of 1,820 children born and living in the city of Banja Luka during the period of 2011 to 2012. The study was conducted in the Public Health Center of Banja Luka. Necessary approval of the General Director and Ethical Committee of the Public Health Center of Banja Luka was obtained before the initiation of research.

Inclusion criteria of the study were: the presence of at least two fully erupted primary teeth in a healthy child and parental consent for participation. Respondents were selected randomly. Healthy children who were visiting regular pediatric checkups in Public Health Center of Banja Luka were referred to the specialist of pediatric and preventive dentistry for the evaluation of risk factors for Severe Early Childhood Caries. First dental visits of children were conducted in a separate office near the pediatric ambulance of Public Health Center. Every child was examined and parent interviewed, but only children who passed the inclusion criteria were included in further research.

Before dental examination of a child, a parent/care-giver was interviewed. A total of 192 questionnaires was filled and subsequently analyzed. Interviews were conducted "face to face" by a single researcher. Questionnaire consisted of two separate parts: the first contained questions related to general information of patient, socio-demographic information, dietary information such as breastfeeding and use of bottles. The second part was Dental record. Issues related to eating habits (breastfeeding and use of bottle) as possible risk factors for S-ECC were considered for the period after primary tooth eruption. Milk bottle referred to infant formula or animal milk (cow milk), and other sweetened content involved juices, milk porridge or sweetened tea. Research questionnaire

was formed according to the guidelines of the American Academy of Pediatric Dentistry (AAPD) [16].

After completion of the interview, dental examination of children was conducted in the office with good natural light. Dental examinations were done in "knee to knee" position where parent and dentist seated opposite to each other so the child was lying with body and legs on the parent's lap, while the head was on the dentist's lap [17]. Oral examination was done using dental mirror and CPI periodontal probe (CPI-Community Periodontal Index). Sterile gauze swabs were used to dry the teeth, and remove dental biofilm. Carious changes on infant's teeth were diagnosed using the International Caries Detection and Assessment System (ICDAS), which is used for diagnosis and records of initial "white spot" carious lesions (without formed holes) to observable carious cavity [18]. Every surface of the tooth was examined and recorded in the second part of the Research Form. Only teeth that were fully present in one's mouth and all crown surfaces visible were taken into consideration.

After data collecting respondents were divided into two groups for the purpose of further analysis and assessment of S-ECCC risk factors. The first group consisted of children who had S-ECC (children with at least one active initial carious lesions on smooth surfaces of maxillary anterior teeth, according to AAPD) and the second group included completely healthy individuals without caries lesions (caries-free children) [10].

For statistical analysis and presentation of results SPSS 16.0 for Windows, MS Office Word and Microsoft Office Excel were used. The results were analyzed statistically by the Fisher's exact test,  $\chi^2$  test (for checking linear growth), and binary logistic regression. Values of  $p < 0.05$  were considered statistically significant.

## RESULTS

A total of 192 children were examined, 99 (51.6%) boys and 93 (48.4%) girls up to 24 months of age. The prevalence of S-ECC in the sample was 34.9%.

Socio-demographic characteristics of families are presented in the Table 1. Majority of mothers ( $n = 117$ ) had secondary school education or lower, which was significant for the occurrence of S-ECC. About 71.87% of fathers also had secondary or lower school education, but this was not significant for the prevalence of caries in the examined sample. Monthly income below 1600 KM - Convertible Marks (< 818,06 euro) reported 79.69% of families.

Most of examined children (89.58%) were breastfed for at least one month ( $n = 172$ ). There was no statistically significant difference in the S-ECC presence between children who were breastfed (34.3%) or not (40.0%) (Table 2). The children who were breast-fed four times per day or more ( $n = 41$ ) were affected more by S-ECC (36.6%) compared to those who had lower frequency of daily feedings (33.6%) but this was not significant (Table 2). A half of respondents (50%) who were breast-fed during the night had S-ECC. About 73.3% of those with S-ECC were breast-fed more than two times during the night. This

**Table 1.** Socio-demographic characteristics of the tested sample  
**Tabela 1.** Sociodemografske karakteristike testiranog uzorka

Variable Vrijednost	S-ECC Cirkularni karijes n (%)	Caries-free Bez karijesa n (%)	P <sup>a</sup>	
<i>Education of mothers</i> <i>Obrazovanje majki</i>				
High school or lower (n=117) Srednja škola ili niže obrazovanje	49 (41.9)	68 (58.1)	.013*	
University education (n=75) Univerzitetsko obrazovanje	18 (24.0)	57 (76.0)		
<i>Education of fathers</i> <i>Obrazovanje očeva</i>				
High school or lower (n=138) Srednja škola ili niže obrazovanje	53 (38.4)	85 (61.6)	.130	
University education (n=54) Univerzitetsko obrazovanje	14 (25.9)	40 (74.1)		
<i>Monthly family income</i> <i>Mesečna primanja porodice</i>				
< 1600 KM (< 818.06 euro) (n=153)	62 (32.8)	91 (67.2)	<.001*	
≥ 1600 KM (≥ 818.06 euro) (n=39)	5 (12.8)	34 (87.2)		
Fisher exact test (Education of mothers, Education of fathers), $\chi^2$ test to check the linear growth (monthly income)				
*Statistically significant results (p<0.05) Fišerov egzaktni test (obrazovanje majke, obrazovanje oca), $\chi^2$ test za proveru linearog rasta (Mesečna primanja) *Statistički značajan rezultat (p<0.05)				

was statistically significant (Table 2). S-ECC was present in 50% of children practicing bedtime baby bottle with milk (Table 3). Children who consumed sweetened liquid (64.7%) or milk (54.2%) in bottle during nighttime were also significantly more affected by S-ECC (Table 3).

## DISCUSSION

The prevalence of ECC varies from 1% to 70% in different parts of the world and can be affected by many factors [2, 3, 19, 20]. A survey conducted in Banja Luka from 2008 to 2010, among 2 to 6 year-old children attending the Centre for pre-school education of Banja Luka, demonstrated high prevalence of dental caries (35.35 %) where carious lesion was diagnosed on a level of already formed carious cavity. The largest percentage of affected teeth (92.69%) was not treated [21]. However, this study did not cover children at infancy (up to 24 months-old) that is from the point of primary prevention the most important and initial caries lesions were not registered either. Leong et al. confirmed that factors occurring in the first year of life strongly influence the experience of early teeth decay, which particularly can be related to the eating habits of a child [9].

The prevalence of caries lesions in our research was high and included 34.9% of all respondents. Most of children came from families whose incomes were below average, considering that even 79.68% of the surveyed families had a monthly income less than 1600 KM (below 818.06 euro). According to the data of the Republic of Srpska Institute of Statistics the average monthly net salary in the Republic of Srpska was around 825 KM (421.81 euro) [22]. This study shows a significant association between

**Table 2.** Distribution of children according to breastfeeding characteristics and appearance of S-ECC  
**Tabela 2.** Klasifikacija dece na osnovu karakteristika dojenja i pojave cirkularnog karijesa

Variable Vrijednost	S- ECC Cirkularni karijes n (%)	Caries-free Bez karijesa n (%)	P <sup>a</sup>	
<i>Breastfeeding (at least one month)</i> <i>Dojenje (najmanje jedan mesec)</i>				
Yes (n=172) Da	59 (34.3)	113 (65.7)	.626	
No (n=20) Ne	8 (40.0)	12 (60.0)		
<i>Duration of breastfeeding</i> <i>Uzrast do kojeg je trajalo dojenje</i>				
The average age of child in months (SD)	7.9 (6.3)	6.7 (4.6)	.147	
Prosečno trajanje u mesecima (SD)				
<i>Breastfeeding – daily frequency</i> <i>Doenje – dnevna učestalost</i>				
<4 times daily (n=131) <4 puta dnevno	44 (33.6)	87 (66.4)	.711	
≥4 times daily (n=41) ≥4 puta dnevno	15 (36.6)	26 (63.4)		
<i>Breastfeeding at night</i> <i>Doenje u toku noći</i>				
Yes (n=58) Da	29 (50.0)	29 (50.0)	.005*	
No (n=134) Ne	38 (28.4)	96 (71.6)		
<i>Breastfeeding at night – frequency</i> <i>Doenje u toku noći – učestalost</i>				
Once (n=30) Jedanput	12 (40.0)	18 (60.0)	.037*	
Twice (n=13) Dvaput	7 (53.8)	6 (46.2)		
Three times or more (n=15) Tri puta ili više	11 (73.3)	4 (26.7)		
<i>Duration of breastfeeding at night</i> <i>Uzrast do kojeg je trajalo dojenje tokom noći</i>				
The average age of child in months (SD)	10.3 (2.6)	12.8 (3.8)	.012*	
Prosečno trajanje u mesecima (SD)				
Fisher exact test (breastfeeding, breastfeeding at night), $\chi^2$ test to check the linear growth (frequency of nighttime breastfeeding), binary logistic regression (Duration of breastfeeding, Duration of breastfeeding at night)				
*Statistically significant results (p<0.05) Fišerov egzaktni test (dojenje, dojenje noću), $\chi^2$ test za proveru linearog rasta (učestalost noćnog dojenja), binarna logistička regresija (uzrast do kojeg je dete dojeno, uzrast do kojeg je dojeno noću) *Statistički značajan rezultat (p<0.05)				

the occurrence of S-ECC and socio-economic status of the family, which is consistent with previous studies [2, 9, 23].

Studies of various researchers have shown that milk and dairy products have multiple benefits for oral health [24, 25]. Their role in prevention of dental pathology, such as caries and periodontal disease is well documented. It has been showed that milk contains a variety of bioactive peptides, as well calcium that play a key role in maintaining good health of teeth [24, 25]. Recent study, among preschoolers, shows that children who were not breast-fed were at increased risk for the development of hypoplastic enamel changes [26]. It has also been found that the act of sucking of mother's milk greatly contributes to the proper formation of the stomatognathic system of infants [25].

**Table 3.** Distribution of children according to the use of baby bottle and appearance of S-ECC  
**Tabela 3.** Klasifikacija dece na osnovu upotrebe boćice i pojave cirkularnog karijesa

Variable Vrijednost	S-ECC Cirkularni karijes n (%)	Caries-free Bez karijesa n (%)	P <sup>a</sup>	
<i>Bedtime use of baby bottle with sweetened liquid (juice, milk porridge, tea) Upotreba boćice sa zasladenom tečnošću tokom uspavljivanja (sok, mleko sa keksom, čaj)</i>				
Yes (n=8) Da	5 (62.5)	3 (37.5)	.130	
No (n=184) Ne	62 (33.7)	122 (66.3)		
<i>Bedtime use of baby bottle with milk Upotreba boćice sa mlekom tokom uspavljivanja</i>				
Yes (n=34) Da	17 (50.0)	17 (50.0)	.049*	
No (n=158) Ne	50 (31.6)	108 (68.4)		
<i>Baby bottle with sweetened liquid during the night (juice, milk porridge, sweet tea) Upotreba boćice sa zasladenom tečnošću tokom noći (sok, mleko sa keksom, čaj)</i>				
Yes (n=17) Da	11 (64.7)	6 (35.3)	.014*	
No (n=175) Ne	56 (32.0)	119 (68.0)		
<i>Baby bottle with milk during the night Upotreba boćice sa mlekom tokom noći</i>				
Yes (n=24) Da	13 (54.2)	11 (45.8)	.041*	
No (n=168) Ne	54 (32.1)	114 (67.9)		
Fisher exact test				
*Statistically significant results (p<0.05)				
Fišerov egzaktni test				
*Statistički značajan rezultat (p<0.05)				

Besides undoubtedly great positive impact on oral health in the literature, there is evidence that human milk under certain conditions, like frequent (on demand) or nighttime feedings after the eruption of primary teeth, leads to acidogenic and cariogenic conditions that contribute to S-ECC [27, 28]. In our study, breastfeeding was not systematically related to the prevalence of S-ECC in the examined children, even frequent daily feedings (more than four times a day) after the eruption of the first primary tooth. Iida et al. found no relationship between breastfeeding, or duration of this habit with the prevalence of S-ECC in children aged two to five years of Mexican-American origin [29]. Nilza and Manoel Ribeiro found no reliable scientific evidence to support the hypothesis of human milk cariogenicity, because it is very complex relationship often masked with other potentially cariogenic variables [13]. On the other hand Li et al. and Matea et al. in their research reported significant association between prolonged and frequent breastfeeding on demand and S-ECC [2, 12]. In the further course of our study we showed that frequent nighttime breastfeeding (or in general practice of nighttime breastfeeding) significantly led to the emergence of S-ECC. Azavedo et al. also showed a link between breastfeeding habits at night (after

the age of 12 months) and higher prevalence of S-ECC on a sample of preschool children [23]. Vachirarojisan et al. reported the prevalence of S-ECC of 58.4% in children 6 to 19 month-old in Thailand. He also showed a significant association between disease and prolonged and frequent breastfeeding, low-income parents, low level of mother's education, high levels of *Streptococcus mutans* in the mouth, and practicing night meal with bottles [3]. Santos and Soviero in children under 36 months old showed the prevalence of dental caries (including "white spots") was 41.6%. They found significant association between the incidence of dental caries and socio-economic status and practicing night meal (bottle or breast feeding) [20].

A large number of children in our study have been using a baby bottle with milk or other sweetened content at bedtime and during the night and that was significantly associated with the occurrence of S-ECC. This is an indicator of poor oral health education of parents who are not aware about harmful effects of such habits. Therefore we believe there is need for introducing obligatory oral health education of parents. Also dental visit of a child around 12 months of age should become legal obligation in Banja Luka and throughout Republic of Srpska. The American Academy of Pediatric Dentistry (AAPD) and the European Academy of Pediatric Dentistry (EAPD) suggest that regular first dental examination of a child should be done no later than one year of life [10, 30]. In the scientific literature there is evidence that suggests that counseling and education of parents about nutrition, oral hygiene and other risk factors contribute to lower prevalence of S-ECC [31].

## CONCLUSION

The prevalence of S-ECC in examined children up to 24 month-old in Banja Luka is high and it is associated with nighttime breastfeeding, use of baby bottle with milk during bedtime/nighttime and use of baby bottle with other sweetened content during nighttime after eruption of the first primary teeth. It is necessary to introduce mandatory first dental examination of a child around the first year of life as well as to organize oral health-education of parents.

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Received: 14.06.2016 • Accepted: 23.08.2016

# Povezanost navika u ishrani sa pojavom cirkularnog karijesa u uzrastu dece do 24 meseca

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## KRATAK SADRŽAJ

**Uvod** Ishrana dece u prve dve godine života bazirana je uglavnom na čestim, tečnim, kašastim i neretko zaslăđenim obrocima, koji nažalost mogu biti dobra podloga za razvoj cirkularnog karijesa. Cilj istraživanja je bio da se ispita uticaj navika u ishrani na pojavu karijesa ranog detinjstva, u uzrastu dece do 24 meseca života u Banjoj Luci u Bosni i Hercegovini.

**Materijal i metode** Istraživanje je sprovedeno po principu analitičke studije preseka, koja je obuhvatala reprezentativni uzorak od 192 deteta. Pre stomatološkog pregleda, roditelj (staratelj) je anketiran o generalijama, sociodemografskim karakteristikama porodice i prehrambenim navikama deteta. Anketa je sprovedena u vidu intervjua („licem u lice“). Ispitanici su podeljeni u dve grupe: prva grupa – deca sa cirkularnim karijesom, i druga grupa – deca bez karijesa. Za statističku analizu i prezentovanje rezultata korišćen je SPSS 16.0 za Windows, MS Office Word i MS Office Excel.

**Rezultati** U ispitivanoj grupi 34,9% dece je obolelo od cirkularnog karijesa. Ispitivanjem navika u ishrani nađeno je da deca koja su dojena noću nakon nicanja prvog zuba u 50% slučajeva imaju KRD, što je bilo statistički značajno ( $p = 0,005$ ). Kao značajan faktor rizika identifikovana je i upotreba boćice sa mlekom ili drugim zaslăđenim sadržajem tokom uspavljivanja i u toku noći ( $p < 0,05$ ).

**Zaključak** Navika noćnog dojenja, te upotrebe boćice sa mlekom prilikom uspavljivanja/tokom noći ili boćice sa drugim zaslăđenim sadržajem u toku noći, a nakon nicanja prvog mlečnog zuba, veoma su značajni faktori rizika za pojavu cirkularnog karijesa kod ispitivane dece.

**Ključne reči:** dojenje; cirkularni karijes; noćno dojenje; boćica; faktori rizika

## UVOD

Rano detinjstvo je ključno za razvoj i unapređenje oralnog zdravlja. Karijes ranog detinjstva (KRD) veoma je značajan i jako veliki javni zdravstveni problem u svetu [1–7]. Prema istraživanjima, to je jedno od najčešćih hroničnih zaraznih oboljenja u detinjstvu, koje je zbog multifaktorijske etiologije veoma teško kontrolisati [8, 9].

Agresivnija forma KRD – cirkularni karijes (Severe early childhood caries – SECC) može se javiti čim se Zub pojavi u ustima, sa lokalizacijom na karijes imunim mestima, odnosno glatkim površinama zuba [10]. Oboljenje započinje kao kredasto beličasta promena na vestibularnoj površini gledi uz gingivu, mada neretko i na oralnoj strani maksilarnih mlečnih sekutića. Razaranje zuba može dosta brzo da napreduje i zahvati ostale grupe zuba. Ukoliko se detetu ne pruži odgovarajuća terapija, oboljenje napreduje ka pulpi, širi se i dovodi do pojave bola i drugih komplikacija.

Kao i ostale forme karijesa, cirkularni karijes se razvija interakcijom tri glavna faktora, i to prisustvom kariogenih mikroorganizama, fermentabilnih ugljikohidrata i zuba, tokom određenog vremenskog perioda [11]. U obzir se moraju uzeti i specifičnosti koje su vezane za rani uzrast, kao što je nezreo odbrambeni sistem, tek iznikli zubi koji su u fazi posteruptivne mineralizacije, hipoplastične promene, poseban vid ishrane (pretežno tečna bazirana na podojima ili upotrebi mlečne formule putem flašice) [12, 13]. Pored navedenih, postoji još čitav niz faktora rizika koji na indirektan način doprinose obolenju mlečnih zuba, a mnogi se još uvek istražuju [8–10].

Dojenje predstavlja prirodan, poželjan način ishrane za novorođenče i nesumljivo ima veliki značaj za celokupno zdravlje i razvoj. Majčino mleko je specifično i po sastavu idealno odgovara ljudskoj vrsti, dok se zamene različitog porekla (adaptirane mlečne formule, mleko životinjskog porekla) umnogome razlikuju po sadržaju. Dojenje ima višestruki značaj za pravilan rast,

obezbeđuje optimalne nutritivne potrebe, imunološku zaštitu dojenčeta, a takođe i minimizira ekonomski uticaj na porodicu. Od strane Svetske zdravstvene organizacije preporučeno je do 24 meseca života [14]. Međutim, Američka akademija za dečju stomatologiju (American Academy of Pediatric Dentistry – AAPD) preporučuje da se sa dojenjem prestane oko 12. meseca života deteta, pa čak i ranije – nakon što prvi Zub nikne (oko šestog meseca), jer ova navika predstavlja rizik za razvoj cirkularnog karijesa (S-ECC) [10].

Ishrana dece u prve dve godine zasnovana je uglavnom na čestim, tečnim, kašastim i neretko zaslăđenim obrocima. Posebnu ulogu u etiologiji karijesa u ranom detinjstvu ima neodgovarajuća upotreba boćice i njena prolongirana upotreba tokom noćnog sna, odnosno njen dodatno zaslăđen sadržaj. Kada dete zaspri, tečnost se razliva po maksilarnim sekutićima, predstavljajući izvanrednu podlogu za razvoj kariogenih bakterija, naročito tokom noći, kada se smanjuje lučenje pljuvačke i izostaje njena puferska, antimikrobnja i uloga samočišćenja [15].

U Banjoj Luci, pa i celoj Republici Srpskoj, ne postoji organizovani preventivni program u stomatologiji. Ne postoji sistematizovan zdravstveno-vaspitni rad sa roditeljima, nedostaju podaci vezani za stanje u oralnom zdravlju i faktorima rizika u najranijem uzrastu, jer ni praksa prvog redovnog stomatološkog pregleda oko godine dana nije utemeljena. Prvi stomatološki pregled je obavezan tek pri upisu u osnovnu školu.

Cilj ovog rada je bio da se utvrdi uticaj navika u ishrani na pojavu karijesa ranog detinjstva, u uzrastu dece do 24 meseca života u Banjoj Luci, Bosna i Hercegovina.

## METODE RADA

Istraživanje je sprovedeno među decom uzrasta do 24 meseca, u periodu između jula 2012. godine i jula 2013. godine. Ispitanje je realizovano kao retrospektivna studija preseka, koja je

obuhvatila 192 deteta oba pola. Reprezentativni uzorak je činilo 10% (nešto više) dece od 1820 rođenih i nastanjenih tokom 2011/2012. godine u gradu Banja Luka. Istraživanje je sprovedeno u Javnoj ustanovi Dom zdravlja Banja Luka. Pre započinjanja istraživanja dobijena je potrebna saglasnost direktora i Etičkog odbora Doma zdravlja Banja Luka. Uslovi za uključivanje ispitanika u istraživanje su bili prisustvo najmanje dva u potpunosti iznikl mlečna zuba kod zdravog deteta, kao i pristanak roditelja za učešće. Ispitanici su odabrani metodom slučajnog izbora. Zdrava deca koja su posećivala redovne pedijatrijske kontrole u Savetovalištu za decu i vakcinisana u Vakcinacionom centru Doma zdravlja Banja Luka upućivana su na prvi stomatološki pregled kod specijaliste dečje i preventivne stomatologije, te evaluaciju faktora rizika za nastanak karijesa ranog detinjstva. Prvi stomatološki pregled dece je vršen u posebnoj ambulanti u sklopu Savetovališta od strane jednog istraživača. Svako dete koje je preporučeno od strane pedijatra je pregledano, ali jedino ona koja su prošla inkluzioni kriterijum bila su uključena u dalji tok istraživanja.

Pre stomatološkog pregleda, roditelj (staratelj) je anketiran. Popunjeno je i naknadno analizirano ukupno 192 ankete. Anketa je sprovedena u vidu intervjua („licem u lice“) od strane jednog istraživača. Za potrebe ovog ispitivanja formiran je Istraživački obrazac koji je sadržao dva dela. U prvom delu su se nalazila pitanja vezana za generalije pacijenta, sociodemografske podatke, dijetetske podatke kao što je dojenje i upotreba flašice. Drugi deo bio je predviđen za evidentiranje zdravstvenog stanja zuba ispitanika. Pitanja vezana za prehrambene navike (dojenje i upotreba flašice) kao moguće faktore rizika za nastanak cirkularnog karijesa odnosila su se za period nakon nicanja prvog mlečnog zuba. Mleko u bočici se odnosilo na mlečne formule ili mleko životinjskog porekla (kravljie), a ostali zasladieni sadržaj je podrazumevao sokove, mleko sa keksom, zasladieni čaj ili kompot. Istraživački obrazac je formiran prema smernicama Američke akademije za dečju stomatologiju (AAPD) [16].

Nakon kompletiranja upitnika vršeni su stomatološki pregledi dece u za to predviđenoj prostoriji sa dobrim prirodnim osvetljenjem. Pošto se radilo o sasvim maloj deci, stomatološki pregledi su vršeni tako da dete leži trupom i nogama roditelju u krilu, dok je glava deteta položena stomatologu u krilo (roditelj i stomatolog sede jedan nasuprot drugog, u takozvanoj „knee to knee“ poziciji) [17]. Pregledi su rađeni pomoću stomatološkog ogledalca i CPI parodontološke sonde (CPI – Community Periodontal Index). Sterilni tupferi gaze su korišćeni za posušivanje zuba, te uklanjanje dentalnog biofilma. Status zuba dece je evidentiran u za to predviđeni deo Istraživačkog obrasca. Kariozne promene kod dece su dijagnostikovane pomoću International Caries Detection and Assessment System (ICDAS), koji služi za dijagnostiku i evidenciju karioznih lezija od početnih promena u gledi bez formiranog kaviteta („bele mrlje“) do uočljivih karioznih kavitetata [18]. Svaka površina zuba je pregledana i evidentirana u Istraživački obrazac. Evidentirani su zubi koji su u potpunosti iznikli i čije su sve površine krunice bile vidljive.

Nakon prikupljanja podataka u svrhu dalje analize i procene faktora rizika ispitanici su podeljeni u dve grupe. Prvu grupu su činila deca koja su imala cirkularni karijes (S-ECC), odnosno najmanje jednu aktivnu početnu karioznu leziju na glatkim površinama maksilarnih frontalnih zuba (AAPD) [10], a drugu grupu su činili potpuno zdravi ispitanici bez karijesa (caries-free).

Za statističku analizu i prezentovanje rezultata korišten je SPSS 16.0 za Windows, MS Office Word i MS Office Excel. Dobijeni rezultati su analizirani statistički pomoću Fišerovog egzaktnog testa,  $\chi^2$  testa (za proveru linearног rasta), kao i binarne logističke regresije. Vrednosti  $p < 0,05$  su se smatrале statistički značajnim.

## REZULTATI

Ukupan uzorak je činilo 192 dece uzrasta do 24 meseca (+/- 3 meseca). Pregledano je ukupno 99 (51,6%) dečaka i 93 (48,4%) devojčice. Prevalencija karijesa u ispitivanom uzorku iznosi je 34,9%.

Sociodemografske karakteristike porodice prezentovane su u Tabeli 1. Većina majki ispitivane dece ( $n = 117$ ) imale su srednjoškolsko obrazovanje ili niže, što je bilo značajno za pojavu karioznog oboljenja kod dece. Oko 71,87% očeva ispitivane dece takođe je imalo srednjoškolsko ili niže obrazovanje, a 79,69% porodica imalo je mesečna primanja ispod 1600 KM (< 818,06 evra).

Većina ispitivane dece, 89,58%, hranjena je na prirodan način ( $n = 172$ ) minimalno jedan mesec. Statistički značajnim se nije pokazala razlika u pojavi karioznog oboljenja kod dece koja su dojena (34,3%) ili nisu (40,0%) (Tabela 2).

Ispitivanjem učestalosti dnevnih podoja nakon nicanja prvog zuba pokazano je da su deca koja su dojena četiri puta dnevno ili češće ( $n = 41$ ) u blago većem (ne značajnom) procentu obolela od cirkularnog karijesa (36,6%) u odnosu na decu koja su imala manji broj dnevnih podoja (33,6%).

Oko 50% dece koja su dojena tokom noći imala su karijes (Tabela 2), a 73,3% obolelih od karijesa je dojeno više od dva puta u toku noći. Ova razlika je bila statistički značajna.

Pojava karijesa u zavisnosti od uspavljivanja i pijenja iz bočice tokom noći je prezentovana u Tabeli 3, gde se vidi da 50% dece koja se uspavljuju pijenjem mleka iz flašice imaju karijes. Deca koja su konzumirala flašicu sa zasladienom tečnošću (64,7%) ili mlekom (54,2%) tokom noći takođe su u značajno većem procentu imala kariozno oboljenje.

## DISKUSIJA

Prevalencija karijesa ranog detinjstva se kreće od 1% do 70% u različitim delovima sveta i na nju utiču mnogi faktori [2, 3, 19, 20]. Istraživanje rađeno u Banjoj Luci od 2008. do 2010. godine na deci koja pohađaju Centar za predškolsko obrazovanje i vaspitanje, gde su u studiji bila uključena deca uzrasta od dve do šest godina, pokazuje visoku prevalenciju karijesa, koja je iznosila 35,35% (gde je karijes dijagnostikovan u formi već formiranog karioznog kavitetata). Najveći procenat obolelih zuba je bio nesaniran, čak 92,69% [21]. Međutim, u ovom istraživanju nije obuhvaćen rani uzrast (do 24 meseca života), koji je sa stanovišta primarne prevencije najvažniji, a ni početne kariozne lezije nisu bile registrovane.

Leong i saradnici potvrđuju da činioči koji se javljaju u prvoj godini života snažno utiču na iskustvo vezano za rani karijes, što se između ostalog posebno odnosi na navike u ishrani deteta [9].

Prevalencija cirkularnog karijesa u ovom istraživanju izuzetno je visoka i iznosi 34,9% svih ispitanika starosti do 24 meseca.

Većina dece je poticala iz porodica čija su primanja ispod prosečnih, s obzirom na to da je čak 79,68% ispitivanih porodica imalo mesečna primanja manja od 1600 KM (ispod 818,06 evra), dok je prema podacima Republičkog zavoda za statistiku RS prosečna mesečna neto plata u Republici Srpskoj iznosila oko 825 KM (421,81 evra) [22]. Ovo istraživanje pokazuje značajnu vezu između pojave cirkularnog karijesa i materijalnog statusa porodice, što je u skladu sa ranijim istraživanjima [2, 9, 23].

Studije različitih istraživača su pokazale da mleko i mlečni proizvodi imaju višestruku korist za oralno zdravlje [24, 25]. Dokazana je njihova uloga u sprečavanju stomatološke patologije, kao što su karijes i parodontopatije, time što se u mleku nalaze razni bioaktivni peptidi, kao i kalcijum, koji imaju ključnu funkciju u održavanju zdravlja zuba [24, 25]. Novije istraživanje na deci predškolskog uzrasta pokazuje da deca koja nisu hranjena majčinim mlekom imaju veći rizik za razvoj hipoplastičnih promena u gleđi [26]. Takođe, akt sisanja u velikoj meri doprinosi pravilnom formirajući stomatognatnog sistema dojenčeta [25]. Međutim, pored nesumnjivo velikog značaja za oralno zdravlje, u literaturi postoje dokazi da majčino mleko u određenim uslovima čestih (na zahtev) ili noćnih podoja nakon nicanja zuba dovodi do stvaranja acidogenih i kariogenih uslova koji doprinose nastanku cirkularnog karijesa [27, 28].

U ovom istraživanju dojenje nije bilo sistematski povezano sa pojavom cirkularnog karijesa kod ispitivane dece, čak ni učestaliji dnevni podoji (više od četiri puta dnevno) nakon nicanja prvog mlečnog zuba.

Iida i saradnici ne nalaze vezu između dojenja, niti dužine trajanja ove navike sa pojmom cirkularnog karijesa kod dece uzrasta od dve do pet godina meksičko-američkog porekla [29]. Nilza i Manoel Ribiero potvrđuju da ne postoji pouzdani naučni dokazi koji podržavaju hipotezu o kariogenosti humanog mleka, te da je u pitanju vrlo kompleksna veza koja je često maskirana mnoštvom drugih potencijalno kariogenih varijabli [13].

Sa druge strane, Li i saradnici, a takođe Matee i saradnici u svojim istraživanjima nalaze vrlo značajnu vezu između prolongiranog i frekventnog dojenja deteta na zahtev i pojave cirkularnog karijesa [2, 12].

U daljem toku ovog istraživanja je pokazano da su u slučaju dojenja tokom noći (nakon nicanja prvog zuba) sve tri ispitivane varijable ukazale na to da je češće noćno dojenje (ili uopšte, praksa noćnog dojenja) značajno dovodila do pojave cirkularnog karijesa kod ispitivane dece.

Azavedo i saradnici takođe pokazuju vezu između navike dojenja noću (nakon navršenih 12 meseci) i češće pojave cirkularnog karijesa na uzorku dece predškolskog uzrasta [23].

Vachirarojsan i saradnici u oblasti U-thong, Tajland, na uzrastu dece od 6 do 19 meseci nalaze pojavu cirkularnog karijesa u procentu od 58,4%, te takođe pokazuju značajnu povezanost oboljenja sa prolongiranim i čestim dojenjem, niskim primanjima roditelja, niskim stepenom obrazovanja majke, visokim nivoom Streptococcus mutans u ustima, te praksom noćnog obroka putem flašice [3].

Santos i Soviero kod dece uzrasta do 36 meseci ukazuju na prevalenciju karijesa (uključujući i „bele mrlje“) od 41,6%. Nisu pronašli značajnu povezanost između učestalosti karijesa i socioekonomskog statusa, odnosno praktikovanja noćnog obroka (flašicom ili dojenjem) [20].

Veliki je broj dece koji je za uspavljanje ili tokom noći koristio flašicu sa mlekom ili drugim zaslađenim sadržajem, što ukazuje na needukovanost roditelja o štetnosti takvih navika, koje su značajno bile povezane sa nastankom cirkularnog karijesa. Na osnovu ovih nalaza je važno naglasiti da postoji neophodnost oralno-zdravstvenog vaspitanja roditelja, odnosno utvrđivanje zakonski obavezognog prvog stomatološkog pregleda deteta oko 12. meseca života u Banjoj Luci, ali i celoj Republici Srpskoj.

American Academy of Pediatric Dentistry – AAPD i European Academy of Pediatric Dentistry – EAPD ukazuju da redovan prvi stomatološki pregled deteta treba obaviti najkasnije do navršenih godinu dana života [10, 30]. U naučnoj literaturi postoje dokazi koji sugerisu da savetovanje i upoznavanje roditelja o pravilnim navikama u ishrani, oralnoj higijeni, kao i rizicima za nastanak karijesa ranog detinjstva doprinose manjoj stopi pojave oboljenja [31].

## ZAKLJUČAK

Prevalencija cirkularnog karijesa dece uzrasta do 24 meseca u Banjoj Luci je visoka. Navika noćnog dojenja i korišćenja flašice sa mlekom u svrhu uspavljanja i tokom noći, kao i upotreba flašice sa zaslađenim sadržajem tokom noći nakon nicanja prvog mlečnog zuba je značajan faktor rizika za pojavu cirkularnog karijesa. Neophodno je uvođenje obavezognog prvog stomatološkog pregleda deteta do godine dana i organizovanje zdravstveno-vaspitnog rada sa roditeljima.

# Dental pulp pain in young and postmenopausal women: a pilot study

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## SUMMARY

**Introduction** This pilot study was aimed to compare pulpal pain provoked by electrical and thermal (cold) stimuli in healthy young women during various phases of menstrual cycle and postmenopausal women.

**Material and methods** The study included 20 regularly menstruating healthy women and 20 postmenopausal women. Electrical (electrical pulp tester) and cold (refrigerant spray) stimuli were performed on mandibular central incisors, twice in regularly menstruating (menstrual and luteal phases) and once in postmenopausal women. Results were expressed as pain threshold values for electrical pulp stimulation (0-80 units) and pain intensity scores (visual numeric scale, from 0 to 10) for cold stimulation.

**Results** In young women, higher pain electrical threshold ( $p=0.484$ ) and pain sensitivity score ( $p=0.015$ ) were observed in luteal in comparison to menstrual phase. In postmenopausal women, electrical pain threshold was significantly higher while pain intensity score was significantly lower than in young women, regardless of the menstrual phase and painful stimuli.

**Conclusion** Lower responsiveness to dental pulp pain was obtained in young women in luteal phase and postmenopausal women.

**Keywords:** dental pulp; menstrual cycle; pain; menopause

## INTRODUCTION

A large number of clinical and experimental studies on sex differences in various painful conditions have shown their higher prevalence in women [1, 2]. Migraine headache, tension-type headache, fibromyalgia, rheumatoid arthritis, osteoarthritis, irritable bowel syndrome, and temporomandibular disorders (TMD) are more common in women than in man [1, 3-8]. Furthermore, women experience higher pain intensity, pain frequency and longer duration of pain [1, 9]. Women are also more likely to consult physicians because of various pain conditions [1]. Sex differences in pain response can be ascribed to social, neurophysiological, genetic, and immunological influences, as well as to the effects of gonadal hormones. A large number of findings suggest gonadal hormones may affect pain perception in women [1, 10]. In the lifetime hormonal levels modulate significant changes in clinical pain conditions in women. Namely, TMD and migraine headache usually appear after puberty and peak during the reproductive period, in the 20-45-age range [10]. Pain intensity in TMD, migraine headache, and other painful clinical conditions increases towards the end of the menstrual cycle and during the first days of menstruation [11, 12]. These findings suggest that decrease in estrogen and progesterone levels during perimenstrual phase cause onset and increase of pain. Hormonal milieu

during early luteal phase (decrease in estrogen concentration with rise in progesterone concentration) has also been related to increased migraine pain intensity. Similarly, TMD pain level can increase during ovulation, the phase characterized by the rapid change in estrogen level [11]. Conversely, pain fluctuation during menstrual cycle has not been observed in patients with fibromyalgia [12].

Changes in pain conditions could be expected after the reproductive period due to both ageing process and other medical causes. Studies have shown that in postmenopausal period the prevalence of TMD and migraines headaches decreases [10], but prevalence of other pain syndromes such as fibromyalgia and osteoarthritis increases [10, 13]. These findings suggest that changes in pain conditions in postmenopausal period depend on the pain modality and characteristics [14]. Unlike clinical pain conditions, it is not yet clear if pain sensitivity on various acute stimuli in healthy women (experimental pain) depends on hormonal status. Namely, several authors have indicated increased pain sensitivity on various stimuli in healthy women during various menstrual phases [15, 16], while others have shown an absence in pain response variability during menstrual cycle [17, 18]. Hormones affect numerous sites for pain sensitivity modulation: primary afferent fibres, spinal cord, brainstem, and cerebrum [19]. As gonadal hormone receptors have been identified throughout the nervous system [20]

it is possible that these hormones affect numerous sites to modulate pain. It has been demonstrated that gonadal hormones interact with nociceptive processes at multiple levels of the peripheral and central nervous system [12, 21]. However, the exact role of gonadal hormones in nociceptive modulation is rather complex and yet not fully understood [22].

Fluctuation in ovarian hormones during menstrual cycle and in various life periods may affect physiological and pathological responses of dental pulp. Namely, estrogen receptors have been detected in the pulp tissue in women in reproductive age, as well as in menopausal women, regardless of age [23]. Also, it has been proven that lower estrogen level during menstrual phase in regularly menstruating and menopausal women is related to lower pulp blood flow [24]. However, according to Tófoli et al. [25], who investigated anaesthetic efficacy and pain induced by dental anaesthesia in regards to menstrual phase, no association between menstrual cycle and investigated parameters of dental anaesthesia has been found, both in healthy regularly menstruating women and women using contraceptives. The influence of hormonal changes during menstrual cycle and postmenopausal period on the pulp pain sensitivity has not yet been investigated. Better insight into these interactions might be of great importance to improve the treatment efficacy.

The aim of this pilot study was to compare the pulp pain response provoked by electrical and thermal (cold) stimuli in young healthy women during various phases of menstrual cycle and postmenopausal women.

## MATERIAL AND METHODS

Forty healthy and pain-free women were selected from patients attending yearly routine check-ups, or subjects accompanying the patients referred to the Specialist Clinic of Faculty of Medicine, University of East Sarajevo, Republic of Srpska, Bosnia and Herzegovina. The subjects were divided into the two groups: the group of regularly menstruating women (menstrual cycle defined as varying between 26 and 28 days), aged 20 to 40 years (mean age  $32.8 \pm 5.9$ ) ( $n=20$ ), and the group of postmenopausal women (at least 2 years after menopause), aged 50 to 65 years (mean age  $59.6 \pm 4.4$ ) ( $n=20$ ). The inclusion criteria were: the presence of vital mandibular central incisor free of caries, restorations, crowns or veneers and tooth wear, without pulp and periodontal disease. Further criteria were no known medical condition, pregnancy, intake of oral contraceptives, any drugs on a regular basis or any medications that could alter pain perception for at least 24 hours proceeding each test session. Furthermore, there was no recent history of orthodontic treatment, periodontal treatment or trauma. Only one tooth per patient was included.

Each regularly menstruating woman was tested twice, once during the menstrual phase (1-5 days of menstrual cycle) and once during the luteal phase (days 17-23). Postmenopausal women were tested once. Dental pulp sensitivity was tested by electrical and thermal (cold) stimuli. Before test the tooth surface was isolated and dried by

cotton rolls. The electric test was done using digital pulp tester (Analytical Technologies, Redmond, WA) with reads from 0-80 units. As a conducting medium, toothpaste was applied lightly to the electrode. The probe was placed on the incisal third of the buccal surface. Participants were asked to raise their hand on first detection of painful sensation. The lowest current intensity that provoked pulp response was considered as pain threshold.

Cold pulpal testing was done by a refrigerant spray (Endo-Frost -50C; Coltene/Whaledent, Altstatten, Switzerland), applied to the incisal third of the buccal surface of the tooth using a cotton pellet for 15 seconds, or until participant indicated a response. Participant was asked to rate her pain on a 0 to 10 numeric rating scale (NRS), with 0 representing no pain and 10 indicating the worst pain the subject has ever experienced. The test was repeated once if there was no response. A 0 value was marked if no response was obtained. A recovery period of at least 2 minutes was allowed after each pulp test.

Data were analyzed using SPSS 19.0 (SPSS, IBM Corp., Armonk, NY). The means and SDs for parametric data, and frequencies for nonparametric data were calculated. To compare pain sensitivity induced by electrical stimulus between the various phases of menstrual cycle and between the two groups of participants Student's t-test for unpaired sample and independent t-test were used, respectively. To compare difference in prevalence and intensity of pain induced by cold stimulus between two phases of menstrual cycle McNemar and Wilcoxon signed-rank tests were used, while intergroup analysis was performed by Chi-square and Mann-Whitney tests. The level of significance was assessed at  $p<0.05$ .

## RESULTS

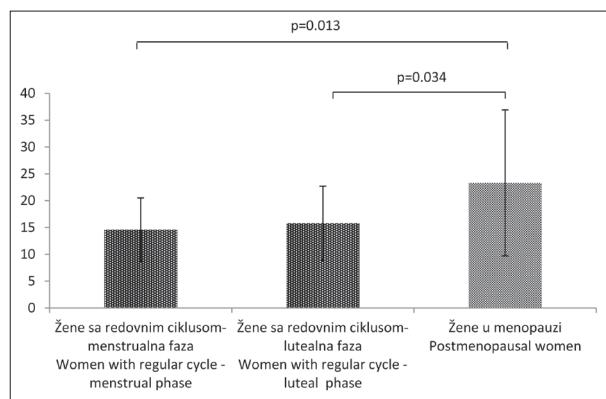
Table 1 shows the frequency of pulp response to the applied stimuli. The reaction of the pulp to electrical stimulus was observed in all subjects and in all periods of measurement. On the other hand, the number of teeth

**Table 1.** Prevalence of dental pulp pain response on electrical and cold stimuli in regularly menstruating and postmenopausal women  
**Tabela 1.** Učestalost reakcije pulpe posle primene električnog i hladnog nadražaja kod žena sa redovnim menstrualnim ciklusom i žena u menopauzi

Group Grupa	Electrical test Elektrotest n (%)	Cold test Test na hladno n (%)
Regularly menstruating women Žene sa redovnim ciklusom		
menstrual phase menstrualna faza	20 (100)	20 (100)
luteal phase lutealna faza	20 (100)	17 (85)
Postmenopausal women Žene u menopauzi	20 (100)	13 (65)*

\* $p = 0.008$  compared to menstrual phase in regularly menstruating women

\* $p = 0.008$  u odnosu na menstrualnu fazu kod žena sa redovnim menstrualnim ciklusom



**Graph 1.** Mean ( $\pm$  SD) of electrical pain threshold in premenopausal and postmenopausal women

**Grafikon 1.** Srednje vrednosti ( $\pm$  SD) praga nadražaja pulpe na električni nadražaj kod žena sa redovnim menstrualnim ciklusom i žena u menopauzi

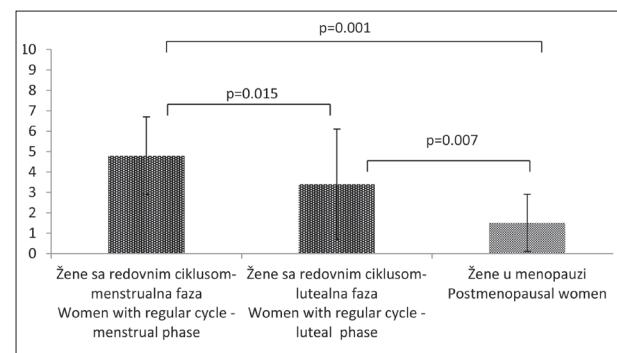
that were responsive to cold stimulation was lower in postmenopausal women (65%) compared to women in menstrual (100%;  $p = 0.008$ ) and luteal phase (85%) of the cycle.

Mean values and standard deviations (SD) of stimulus threshold and intensity of pain in studied groups are shown in Graphs 1 and 2. The sensitivity analysis in the pulp with respect to the cycle phase showed greater pulp sensitivity to electrical (lower threshold level) and cold stimuli (higher pain intensity) in the menstrual compared to the luteal phase, however, the difference was statistically significant only for cold stimulation ( $p = 0.015$ ). The pulp in postmenopausal women showed significantly higher threshold level to electric stimulus and significantly lower pain intensity to cold stimulus compared to women with regular menstrual cycle, regardless of the cycle phase (Graphs 1 and 2).

## DISCUSSION

In order to clarify the possible influence of gonadal hormonal fluctuations on pain sensitivity of the pulp, the current research examined the effects of electric and cold stimuli in women of reproductive age, within two phases of menstrual cycle (menstrual and luteal), and postmenopausal women. The results showed that luteal phase in women with regular menstrual cycle characterized by high levels of gonadal hormones, and menopause - a period of chronic reduction in the level of gonadal hormones, are periods of lower pulp sensitivity.

In our study, pulpal pain was induced by electric and cold stimuli in healthy women without the presence of other painful conditions (experimental pain model). Evaluation of pain sensitivity in healthy subjects is important for investigation of various aspects of mechanisms that underlie pain. Sensory manifestations and sensory-motor interaction of previously clearly defined pain can be determined on experimental pain model. Moreover, experimental pain model is valuable for pharmacological and clinical studies in order to quantify the sensitivity of the nociceptive system in pain patients, as well as to predict



**Graph 2.** Mean ( $\pm$  SD) of pain intensity to cold stimulation in premenopausal and postmenopausal women

**Grafikon 2.** Srednje vrednosti ( $\pm$  SD) intenziteta pulpnog bola na hladni nadražaj kod žena sa redovnim menstrualnim ciklusom i žena u menopauzi

clinical pain and clinical pain outcomes [26]. Research on sensitivity of healthy women to various stimuli during menstrual cycle have not given clear evidence of experimental pain hormonal dependence [12, 27]. Some authors have found that pain perception varies during the cycle [15, 16], while others have not confirmed a link between pain and hormonal fluctuations during the cycle [17, 18]. Discrepancies in results can be attributed to methodological differences, such as type of observed experimental pain, stimulated tissue, a measuring outcomes (threshold or intensity) and functional division of the menstrual cycle with no measurement of gonadal hormone concentrations [12, 27]. An interesting fact is that the brain cortex activity induced by experimental painful stimuli varies between menstrual phases with high levels of estrogen and phase characterized by low hormone levels, and these changes are not always accompanied by altered response to pain [28]. Unlike the experimental pain, it has been shown that pain in certain clinical pain conditions varies during menstrual cycle [10]. In these patients, many other factors can influence the perception of pain during menstrual cycle. Also, patients with pain conditions may have altered sensitivity to hormonal changes during the cycle.

The current study investigated the pulp reaction to electrical and cold stimuli. Electric pulp test and cold test using cold spray are the most frequently used tests for investigation of pulp sensitivity [29]. Various types of stimuli have been applied in experimental studies that investigate the relationship between hormonal status and pain and its characteristics. It has been observed that various stimuli differently activate nociceptive afferent fibres in the tissue. Electric stimuli trigger all classes of afferent neurons, while thermal stimuli only A- $\delta$  and C fibres [30]. Also, certain types of stimuli can cause stress, while others activate endogenous regulatory mechanisms of pain. Therefore, hormonal status can have different effects on different stimuli. In fact, it has been shown that menstrual cycle has opposite effect on pain caused by electrical stimulus from pain caused by other painful stimuli (pressure, cold, warm, ischemic pain) [31]. In our study, higher pulp sensitivity to electrical as well as to cold stimulation was observed in the menstrual phase. Although various groups of nerve fibres contribute in the occurrence of various types of pain, in dentin-pulp complex both stimuli cause

activation of peripherally placed A- $\delta$  fibres that are mediators of sharp, localized pain. Centrally positioned C fibres that are responsible for the appearance of dull, long-term pain were not activated during testing, unless greater intensity of electricity was applied or there was an irritation that caused tissue damage. It is important to note that in addition to the type of stimulation, pain sensitivity varies on the type of tested tissue and the depth of tissue to which the stimulus is applied [15].

In women of reproductive age increased pain sensitivity, especially to cold stimulation, was recorded during menstrual phase, phase characterized by low levels of estrogen and progesterone, while lower sensitivity was present during luteal phase when estrogen and progesterone levels were high. Gonadal hormones, estrogen and progesterone, can express pronociceptive as well as antinociceptive effects depending on the overall hormonal profile [12]. It is believed that at the end of luteal and in early follicular phase, when the levels of both gonadal hormones are low, sympathetic tone, inflammatory response, the synthesis of prostaglandins and affective symptoms reach their highest level, while descending inhibitory systems are at the lowest level [12]. This explains why perimenstrual phase is period of increased sensitivity to various painful conditions. On the other hand, in the middle luteal phase, gonadal hormones reduce pain sensitivity through increased GABAergic tone and increased cortisol secretion [12]. These mechanisms could explain differences in pulp sensitivity during investigated periods of menstrual cycle observed in the current study.

Compared with reproductive age women, in menopausal women sensitivity of pulp was lower, regardless of the applied stimulus and the phase of cycle. Although the influence of gonadal hormones in the reproductive age on clinical pain conditions is somewhat clarified, data on their impact in menopause are different [27]. A study that compared the incidence of various painful conditions in menopausal women with premenopausal period, has found that menopausal changes depend on the type and characteristics of pain [14]. Headaches and cervical / lumbar pain decreased or even disappeared, while developed or intensified joint pain affected more than half of women. Pain intensity and painful area also change with menopause. In most of the cases, high intensity pain that would affect larger area remained unchanged or decreased, while low intensity pain and pain that affects smaller less area increased [14]. The impact of ageing process on the pulp tissue and its sensitivity should not be ignored. Reduction of painful pulp reactions in elderly is attributed to reduction in number of nerve fibres, creation of secondary and tertiary dentin and tubule occlusion [32]. However, significant impact of age on dental pulp response caused by electrical stimulation was not found [33, 34]. Reaction to electrical test was obtained in all subjects, in women of reproductive age as well as menopausal women. On the other hand, it has been shown that the sensitivity of pulp to cold stimuli decreases with age [35]. Unlike electrical test, which is dependent on the ions movement, cold stimulus causes pain response by dentinal fluid movement [36], which can partly ex-

plain reduction of pulp sensitivity to cold in menopausal women. In the current study response to cold stimulation was not obtained in 35% of menopausal women even after two repeated measurements. An interesting finding is that the application of cold spray caused a painful reaction in all subjects in the menstrual phase, but not in 15% of subjects in the luteal phase, what could be misinterpreted in clinical conditions as a loss of tooth vitality. Studies have shown that the response of dental pulp to cold stimulation is the most reliable in this particular age group (21–50 years) [29]. Findings of decreased pain sensitivity in the luteal phase of menstrual cycle characterized by high levels of sex hormones, as well as reduced sensitivity during menopause, a period of chronic reduction in the levels of gonadal hormones, suggest that if there is an association between gonadal hormone levels and pain sensitivity, it is not simple.

Potential variations were not examined in this study. Namely, the division of menstrual cycle in phases was performed on the basis of medical history, without measurement of gonadal hormone concentration. Measuring gonadal hormones levels is important for precise determination of menstrual cycle phase, as well as in determining the correlation between hormonal levels and painful response. However, only a few studies have investigated the influence of gonadal hormone concentration on experimental pain until now. Secondly, sensitivity of the pulp is measured only in the two phases of menstrual cycle. However, to determine the relationship between hormonal status and pain, a research should include more cycle phases.

## CONCLUSION

The results of this pilot study indicate correlation between pulpal pain and menstrual cycle. Higher sensitivity of the pulp to cold stimulus was recorded in the menstrual phase compared to the luteal phase. Pulpal sensitivity in menopausal women was reduced regardless of the type of stimulus and cycle phase.

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Received: 24.06.2016 • Accepted: 31.08.2016

# Pulpni bol kod žena u reproduktivnoj dobi i menopauzi: pilot studija

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## KRATAK SADRŽAJ

**Uvod** Cilj ovog pilot istraživanja je bio da se ispita puljni bol izazvan delovanjem električnog i hladnog nadražaja u različitim fazama menstrualnog ciklusa kod zdravih žena sa redovnim menstrualnim cikusom i da se upoređi sa pulpnim bolom žena u menopauzi.

**Materijal i metode rada** U istraživanje je uključeno 20 zdravih žena sa redovnim menstrualnim ciklusom i 20 zdravih žena u menopauzi. Osetljivost pulpe donjeg centralnog sekutića je određena primenom električnog i hladnog nadražaja. Ispitanice sa redovnim menstrualnim ciklusom su ispitane u toku menstrualne i lutealne faze ciklusa, a žene u menopauzi jednom. Rezultati su prikazani kao prag nadražaja za električni, odnosno intenzitet bola za test na hladno.

**Rezultati** Veći prag nadražaja ( $p = 0,484$ ) i manji intenzitet bola ( $p = 0,015$ ) zabeležen je u lutealnoj u poređenju sa menstrualnom fazom kod žena sa redovnim menstrualnim ciklusom. Kod žena u menopauzi prag nadražaja je značajno veći i intenzitet bola značajno manji u poređenju sa ženama u reproduktivnoj dobi, nezavisno od faze ciklusa i vrste primljenog stimulansa.

**Zaključak** Manja osetljivost pulpe je zabeležena kod žena u lutealnoj fazi ciklusa, kao i kod žena u periodu menopauze.

**Ključne reči:** bol; menopauza; menstrualni ciklus; zubna pulpa

## UVOD

Brojna klinička i eksperimentalna istraživanja o povezanosti pola i različitih bolnih stanja su ukazala na njihovu češću pojavu kod žena [1, 2]. Migrenozne i tenzije glavobolje, fibromialgija, reumatoidni artritis, artritis, sindrom iritabilnog kolona i temporomandibularne disfunkcije (TMD) češće se javljaju kod žena u poređenju sa muškarcima [1, 3–8]. Takođe, kod žena bolovi su jači, učestaliji i dugotrajniji [1, 9]. U odnosu na muškarce, žene se značajno češće javljaju i na terapiju različitih bolnih stanja [1]. Polne razlike u percepciji i u odgovoru na bol se mogu prisati socijalnim, neurofiziološkim, genetskim i imunološkim uticajima, kao i delovanju polnih hormona. Veliki broj nalaza ukazuje na povezanost polnih hormona i bola kod žena [1, 10]. U toku života, značajne promene u kliničkim bolnim stanjima žene su modulirane hormonalnim statusom. Naime, TMD i migrenozne glavobolje se najčešće javljaju posle puberteta, sa najvećom učestalošću između 20. i 45. godine života, odnosno u reproduktivnoj dobi [10]. Bol kod TMD, migrenozni bol, ali i bol kod drugih bolnih stanja se pojačava neposredno pred kraj menstrualnog ciklusa i tokom prvih dana menstruacije [11, 12]. Ovi nalazi ukazuju da snižavanje nivoa estrogena i progesterona tokom perimenstrualne faze izaziva bol, odnosno intenzivira ga. Hormonski milje tokom rane lutealne faze (sniženje nivoa koncentracije estrogena i povećanje nivoa progesterona) takođe se povezuje sa povećanom učestalošću migrenoznih bolova [12]. Slično, bol kod TMD se može ponovo intenzivirati u toku perioda ovulacije, faze brze izmene nivoa estrogena [11]. Sa druge strane, fluktuacije bola tokom menstrualnog ciklusa kod pacijenata sa fibromialgijom nisu zabeležene [12].

Promene u bolnim stanjima se mogu očekivati i posle završetka reproduktivnog doba, kako zbog fiziološkog procesa starenja tako i zbog drugih medicinskih razloga. Istraživanja pokazuju da se učestalost TMD i migrenoznih glavobolja smanjuje posle menopauze [10], ali se povećava učestalost drugih bolnih sindroma kao što su fibromialgija i artritis [10, 13]. Ovi nalazi ukazuju da izmene bolnih stanja posle menopauze zavise od vrste i karakteristika bola [14].

Za razliku od kliničkih bolnih stanja, još uvek nije jasno da li je bolna osetljivost na različite akutne nadražaje kod zdravih žena (eksperimentalni bol) zavisna od hormonalnog statusa. Naime, pojedini autori ukazuju na povećanu osetljivost zdravih žena na nadražaje tokom različitih faza menstrualnog ciklusa [15, 16], dok drugi na odsustvo varijabilnosti u odgovoru na bol tokom ciklusa [17, 18]. Modulacija bola se odvija na više nivoa: primarnim aferentnim vlastinama, kičmenoj moždini, moždanom stablu i mozgu [19]. Receptori za polne horomone se nalaze u nervnom sistemu [20], što ukazuje na mogući uticaj horomona na različite strukture nervnog sistema. Istraživanja su dokazala da polni hormoni interaguju sa nociceptivnim procesom na više nivoa i u perifernom i centralnom nervnom sistemu [12, 21]. Ipak, uloga polnih hormona u nociceptivnoj modulaciji je kompleksna i još uvek nije u potpunosti razjašnjena [22].

Varijacije polnih hormona tokom menstrualnog ciklusa i u različitim periodima života bi mogle da utiču na fiziološke i patološke odgovore zubne pulpe. Naime, dokazano je prisustvo estrogenskih receptora u tkivu pulpe žena u reproduktivnoj dobi, kao i u pulpi žena u menopauzi, koje nije zavisno od starosne dobi [23]. Takođe, pokazano je da je snižen nivo estrogena u menstrualnoj fazi kod žena sa redovnim ciklusom i kod žena u menopauzi povezan sa smanjenim protokom krvi u tkivu pulpe [24]. Međutim, u istraživanju Tófoli i sar. [25], gde je ispitivana povezanost između anestetičke efikasnosti i bola prilikom aplikacije lokalne anestezije sa menstrualnim ciklусом, nije uočen značajan uticaj ciklusa na ispitivane parametre lokalne anestezije kod zdravih žena sa redovnim menstrualnim ciklusom i onih koje uzimaju oralne kontraceptive. Uticaj hormonalnih promena tokom menstrualnog ciklusa i u periodu menopauze na pulpnu bol još uvek nije ispitivan. Poznavanje ovih interakcija bi moglo biti od velike važnosti za efikasnije lečenje pacijenata.

Cilj ovog pilot istraživanja je da se ispita puljni bol izazvan delovanjem električnog i hladnog nadražaja u različitim fazama menstrualnog ciklusa kod zdravih žena sa redovnim cikusom i da se upoređi sa pulpnim bolom žena u menopauzi.

## MATERIJAL I METODE RADA

Ispitivanjem je obuhvaćeno 40 žena koje su se javile u Specijalistički centar Medicinskog fakulteta Univerziteta u Istočnom Sarajevu, Republika Srpska, Bosna i Hercegovina, radi kontrolnog pregleda ili kao pratnja osobama sa zakazanom stomatološkom intervencijom. Ispitanice su podeljene u dve grupe: grupu žena sa redovnim menstrualnim ciklusom (trajanje menstrualnog ciklusa između 26 i 28 dana), starosti od 20 do 40 godina (prosečna starost  $32,8 \pm 5,9$ ) ( $n = 20$ ) i grupu žena u menopauzi (najmanje dve godine od početka menopauze) starosti od 50 do 65 godina (prosečna starost  $59,6 \pm 4,4$ ) ( $n = 20$ ). Kriterijumi za uključivanje u studiju su bili: postojanje klinički intaktnog donjeg centralnog sekutića (bez karijesa, restauracija, fiksne nadoknade i bez znakova zubnog trošenja) bez znakova i simptoma znakova oštećenja pulpe i apeksnog periodoncijuma. Žene sa sistemskim oboljenjima, žene u toku trudnoće, one koje se nalaze na hormonskoj ili drugoj terapiji ili koje su uzimale lekove koji utiču na percepцију bola 24 h pre ispitivanja nisu uključene u istraživanje. U istraživanje nisu uključene ni one ispitnice kod kojih je neposredno pre početka istraživanja završena ortodontska ili parodontološka terapija ili je zabeleženo traumatsko oštećenje zuba. Od svake ispitnice u istraživanje je uključen samo jedan zub.

Ispitanice sa redovnim menstrualnim ciklusom su ispitane dva puta: u toku menstrualne (1–5. dan ciklusa) i lutealne faze (17–23. dan ciklusa) menstrualnog ciklusa, a žene u menopauzi jednom. Osetljivosti pulpe je ispitana na električni i hladni stimulans. Pre ispitivanja Zub je izolovan vaterolnama i posušen. Za ispitivanje osetljivosti na električni nadražaj primenjen je aparat za ispitivanje vitaliteta (Analytical Technologies, Redmond, WA) sa skalom od 0 do 80. Da bi se obezbedio intimniji kontakt elektrode aparata sa površinom zuba, na vrh elektrode je postavljena manja količina paste za zube. Elektroda aparata je postavljena na incizalnu trećinu vestibularne površine zuba. Ispitanice su zamoljene da podizanjem ruke signaliziraju kada prvi put oseće bol. Najmanji intenzitet struje na kome je dobijena reakcija pulpe predstavlja je prag nadražaja. Osetljivost pulpe na hladno je određena primenom hladnog spreja (Endo-Frost-50°C; Coltene/Whaledent, Altstatten, Switzerland), koji je na incizalnu ivicu zuba aplikovan preko vatice i pincete u trajanju od 15 sekundi, odnosno dok ispitnice nisu signalizirale pulpnu reakciju. Ispitanice su zamoljene da odrede intenzitet doživljenog nadražaja (intenzitet bola) na numeričkoj vizuelnoj skali bola, gde vrednost 0 predstavlja odsustvo bola, dok vrednost 10 predstavlja najveći bol koji je ikada doživljen. Vrednost 0 je određena u onim situacijama gde nije zabeležen odgovor pulpe posle ponovljene primene nadražaja, sa pauzom od dva minuta između dva merenja.

Statistička analiza podataka je urađena u statističkom programu SPSS 19.0 (SPSS, IBM Corp., Armonk, NY). Rezultati su prikazani u obliku srednjih vrednosti i standardnih devijacija (SD) za numerička, odnosno učestalosti za atributivna obeležja. Za određivanje razlike u reakciji pulpe na električni nadražaj u različitim fazama menstrualnog ciklusa kao i između grupa korišćen je t-test za vezani uzorak, donosno t-test za dva nezavisna uzorka. Razlike u učestalosti reakcije i intenzitetu bola na hladni nadražaj između dve faze ciklusa su ispitane primenom Mek Nemarovog testa i Vilkoksonovog testa ekvivalentnih parova, a između ispitivanih grupa korišćenjem hi-kvadrat i Man-Vit-

nijevog testa. Vrednosti  $p < 0,05$  su smatrane značajnim u svim analizama.

## REZULTATI

U tabeli 1 prikazana je učestalost reakcije pulpe na primenjene stimulanse. Reakcija pulpe na elektrotest je zabeležena kod svih ispitica i u svim periodima merenja. Sa druge strane, broj zuba koji su reagovali na hladni nadražaj je bio manji kod žena u menopauzi (65%) u poređenju sa ženama u menstrualnoj fazi (100%;  $p = 0,008$ ) i lutealnoj fazi (85%) ciklusa.

Srednje vrednosti i standardne devijacije (SD) praga nadražaja i intenziteta bola u ispitivanim grupama su prikazane na grafikonima 1 i 2. Analiza osetljivosti pulpe u zavisnosti od faze ciklusa pokazuje veću osetljivost pulpe na električni (niži prag nadražaja) i hladni stimulans (veći intenzitet bola) u menstrualnoj u poređenju sa lutealnom fazom, ali razlika je dostigla statističku značajnost samo za nadražaj na hladno ( $p = 0,015$ ). Pulpa kod žena u menopauzi je pokazala značajno veći prag nadražaja na električni stimulans i značajno manji intenzitet bola na hladno u poređenju sa ženama sa redovnim menstrualnim ciklusom, bez obzira na fazu ciklusa (Grafikon 1 i 2).

## DISKUSIJA

Kako bi se razjasnilo mogući uticaj fluktuacije polnih hormona na bolnu osetljivost pulpe, u okviru ovog istraživanja je ispitivan efekat električnog i hladnog stimulansa kod žena u reproduktivnoj dobi, u okviru dve faze menstrualnog ciklusa (menstrualne i lutealne), i kod žena u menopauzi. Dobijeni rezultati su pokazali da lutealna faza kod žena sa redovnim menstrualnim ciklusom, koju karakteriše visok nivo polnih hormona, i menopauza, period hroničnog smanjenja nivoa polnih hormona, jesu periodi manje osetljivosti pulpe.

U ovom istraživanju puljni bol je izazvan delovanjem električnog i hladnog stimulansa kod zdravih žena bez prisutnih drugih bolnih stanja (model eksperimentalnog bola). Određivanje bolne osetljivosti zdravih ispitnika je od važnosti za ispitivanje različitih aspekata mehanizama koji se nalaze u osnovi bola. Na eksperimentalnom modelu bola se mogu odrediti senzorne manifestacije i senzorno-motorne interakcije prethodno jasno definisanog bola. Takođe, eksperimentalni model bola je od važnosti u farmakološkim i kliničkim istraživanjima da bi se odredila osetljivost nociceptivnog sistema kod pacijenata sa bolom, kao i u cilju predviđanja nastanka kliničkih bolnih stanja i njihovih posledica [26]. Istraživanja o osetljivosti zdravih žena na različite nadražaje tokom menstrualnog ciklusa nisu dala jasne dokaze o hormonalnoj zavisnosti eksperimentalnog bola [12, 27]. Pojedini autori nalaze da percepцијa bola varira tokom ciklusa [15, 16], dok drugi ne potvrđuju povezanost bola i hormonskih fluktuacija tokom ciklusa [17, 18]. Neslaganja u rezultatima se većim delom mogu pripisati metodološkim razlikama kao što su vrsta eksperimentalnog bola koji se ispituje, tkivo koje se stimuliše, način merenja bola (prag ili intenzitet) i funkcionalna podela menstrualnog ciklusa bez kvantifikacije nivoa hormona [12, 27]. Interesantan je podatak da se aktivnost korteksma mozga na eksperimentalni bolni stimulans razlikuje između menstrualne faze sa visokim nivoom estrogena i faze

koju karakteriše nizak nivo hormona, i da te promene nisu uvek pružene promjenjenim odgovorom na bol [28]. Za razliku od eksperimentalnog bola, dokazano je da bol kod određenih kliničkih bolnih stanja varira tokom menstrualnog ciklusa [10]. Kod ovih pacijenata brojni drugi faktori mogu da utiču na percepciju bola tokom menstrualnog ciklusa. Takođe, pacijenti sa bolnim stanjima mogu imati izmenjenu osetljivost na hormonalne promene tokom ciklusa.

U ovom istraživanju je ispitivana reakcija pulpe na električnu i hladnu stimulaciju. Elektrotest i test na hladno, primenom hladnog spreja, najčešće su primenjivani testovi za ispitivanje osetljivosti pulpe [29]. Različite vrste stimulansa se primjenjuju u eksperimentalnim istraživanjima koja istražuju povezanost hormonalnog statusa sa bolom i njegovim karakteristikama. Uočeno je da različiti nadražaji različito aktiviraju aferentna nociceptivna vlakna u tkivima. Električni aktiviraju sve klase aferentnih neurona, a termalni stimulansi samo A-δ i C vlakna [30]. Takođe, pojedine vrste nadražaja mogu izazvati stres, dok druge aktiviraju endogene regulatorne mehanizme bola. Stoga, hormonalni status može imati različite efekte na različite stimulanse. Naime, pokazano da menstrualni ciklus ima suprotan efekat na električnim nadražajem izazvanim bolom od bola koji izazivaju drugi bolni stimulansi (pritisak, hladno, toplo i ishemični bolni stimulansi) [31]. U ovom istraživanju veća osetljivost pulpe i na električnu i na hladnu stimulaciju je uočena u menstrualnoj fazi. Iako različite grupe nervnih vlakana doprinose nastanku različitih vrsta bola, oba stimulansa u pulpo-dentinskem kompleksu izazivaju aktivaciju periferno postavljenih A-δ vlakana, koja su medijatori oštrog, lokalizovanog bola. Centralno pozicionirana C vlakna, koja su odgovorna za pojavu tupog, dugotrajnog bola, ne aktiviraju se prilikom testiranja, osim ukoliko se ne primeni struja većeg intenziteta, odnosno nadražaj koji uzrokuje oštećenja tkiva. Važno je pomenuti da pored vrste nadražaja, bolna osetljivost varira u zavisnosti od vrste tkiva koje se ispituje i dubine tkiva na koje se stimulans primjenjuje [15].

Kod žena u reproduktivnoj dobi veća bolna osetljivost, posebno na hladnu stimulaciju, zabeležena je tokom menstrualne faze, faze koju karakteriše nizak nivo estrogena i progesterona, dok je niža osetljivost bila prisutna tokom lutealne faze, kada su koncentracije estrogena i progesterona visoke. Polni hormoni, estrogen i progesteron, mogu da ispolje i pronociceptivne i antinociceptivne efekte, što zavisi od celokupnog hormonskog profila [12]. Smatra se da na kraju lutealne i u ranoj folikularnoj fazi, kada su nivoi oba polna hormona niski, simpatički tonus, inflamatorni odgovor, sinteza prostaglandina i afektivni simptomi dostižu svoj najveći nivo, dok su descendantni inhibitorni sistemi na najnižem nivou [12]. Ovim se objašnjava zašto je perimenstrualna faza vreme povećane osetljivosti za različita bolna stanja. Sa druge strane, u sredini lutealne faze, polni horomoni smanjuju bolnu osetljivost zbog povećanja GABA-neričkog tonusa i pojačanog oslobođanja kortizola [12]. Ovim mehanizmima bi se moglo objasniti razlike u osetljivosti pulpe u ispitivanim fazama menstrualnog ciklusa uočene u ovom istraživanju.

U poređenju sa ženama u reproduktivnoj dobi, kod žena u menopauzi osetljivost pulpe je niža, nezavisno od primjenjenog stimulansa i faze ciklusa. Iako je uticaj polnih hormona u reproduktivnoj dobi na klinička bolna stanja donekle razjašnjen,

podaci o njihovom uticaju u menopauzi su različiti [27]. U istraživanju u kojem je komparirana učestalost različitih bolnih stanja u menopauzi sa periodom pre menopauze, uočeno je da promene koje nastaju u menopauzi zavise od vrste i karakteristika bola [14]. Naime, glavobolje i cervikalni/lumbalni bolovi su se smanjili ili čak nestali, dok je došlo do razvoja ili pojačavanja bola u zglobovima u više od polovine žena. Intenzitet i bolno područje se takođe menjaju sa menopauzom. U većini slučajeva bolovi visokog intenziteta koji su zahvatili veću površinu ostajali su nepromjenjeni ili su se smanjili, dok su se bolovi slabog intenziteta i oni koji su zahvatili manje područje pogoršali [14]. Ne treba zanemariti uticaj samog procesa starenja na tkivo pulpe i njenu osetljivost. Smanjenje bolnih reakcija pulpe kod starijih osoba se pripisuje redukciji broja nervnih vlakana, stvaranju sekundarnog i tercijarnog dentina, kao i okluziji tubula [32]. Ipak, nije pokazan značajan uticaj godina na reakciju zubne pulpe izazvane električnom stimulacijom [33, 34]. Elektrotestom reakcija je dobijena kod svih ispitanica, i žena u reproduktivnoj dobi i žena u menopauzi. Sa druge strane, pokazano je da se osetljivost pulpe na hladno smanjuje sa starenjem [35]. Za razliku od elektrotesta, koji je zavisan od kretanja jona, hladni stimulans izaziva bolnu reakciju kretanjem dentinskog likvora [36], čime se delimično može objasniti smanjenje osetljivosti pulpe na hladno kod žena u menopauzi. Naime, u ovoj studiji kod čak 35% žena u menopauzi nije dobijena reakcija na hladnu stimulaciju ni posle dva ponovljena merenja. Interesantan nalaz je da je primena hladnog spreja izazvala bolnu reakciju kod svih ispitanica u menstrualnoj, ali ne i kod 15% njih u lutealnoj fazi, što bi se u kliničkim uslovima moglo pogrešno interpretirati kao gubitak vitaliteta zuba. Istraživanja pokazuju da je reakcija zubne pulpe na hladnu stimulaciju najpouzdanija baš u ovoj starosnoj dobi (21–50) godina [29]. Uzimajući u obzir postojanje smanjene bolne osetljivosti u lutealnoj fazi menstrualnog ciklusa, koju karakteriše visok nivo polnih hormona, kao i smanjenu bolnu osetljivost tokom menopauze, perioda hroničnog smanjenja nivoa polnih hormona, može se prepostaviti da ako uticaj polnih hormona na bolnu osetljivost i postoji, taj uticaj nije jednostavno linearan.

Veliki broj potencijalnih varijabli nije ispitana u ovom istraživanju. Naime, podela menstrualnog ciklusa na faze je sprovedena na osnovu anamnističkih podataka, bez kvantifikacije polnih hormona. Merenje nivoa polnih hormona je od značaja, kako zbog preciznog određivanja faze menstrualnog ciklusa, tako i za određivanje korelacije između nivoa horomana i bolnog odgovora. Ipak, samo u nekoliko studija do sada je ispitivan uticaj koncentracije polnih hormona na eksperimentalni bol. Takođe, osetljivost pulpe je merena samo u dve faze menstrualnog ciklusa. Ipak, za utvrđivanje povezanosti hormonalnog statusa sa bolom ispitivanje treba da obuhvati veći broj ciklusičkih faza.

## ZAKLJUČAK

Rezultati ove pilot studije ukazuju na povezanost između pulpnog bola i menstrualnog ciklusa. Veća osetljivost pulpe na hladni stimulans je zabeležena u menstrualnoj fazi u poređenju sa lutealnom fazom. U odnosu na žene u reproduktivnoj dobi, osetljivost pulpe kod žena u menopauzi je smanjena, nezavisno od vrste nadražaja i faze ciklusa.

# Fiber reinforced composite bridge as a replacement for missing upper permanent lateral incisor – a case report

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## SUMMARY

Hypodontia of upper lateral incisors is significant aesthetic problem in young people. Ideal solution to this problem would be an implant placement, but there is a problem of unfinished growth and development. It is therefore necessary to have a temporary solution in order to maintain normal stomatognathic system functions and solve aesthetic problems until the final restoration is placed.

The aim of this study was to show the possibility of treating hypodontia of permanent upper lateral incisors by creating adhesive bridge in one visit.

A 15-year-old patient was referred to the Department for Prosthodontics, School of Dental Medicine in Belgrade with hypodontia of permanent upper lateral incisors. The patient had braces until 14 years of age after which she was rehabilitated with partial denture that was neither aesthetically nor functionally acceptable solution for her and significantly affected her emotional security. The adhesive bridge was made using GC composite fibers and a set of GC composite materials for the purpose of temporary replacement of missing teeth. This treatment provided good functional and aesthetic results. The space for the placement of two endosseous implants is preserved and a period of adaptation positively influenced the patient's social development in sensitive adolescent age.

**Keywords:** adhesive bridges; hypodontia of lateral incisors; fiber-reinforced composites

## INTRODUCTION

Hypodontia or the lack of permanent upper lateral incisors is caused by the absence of formation of tooth germs in the jaw and occurs in 20% of population, more often in permanent dentition [1, 2]. It is significant functional and aesthetic problem for young people in adolescence. Treatment with restorations of high aesthetic value is mainly limited by unfinished growth and development of body.

Replacement of missing anterior teeth, especially in children, is a major challenge for clinicians. Therefore, multidisciplinary approach with cooperation of children's dentist, orthodontist, prosthodontist and oral surgeon is of great importance in order to preserve the space and supporting tissues until definitive therapy. Partial dentures are often treatment of choice in children until the eruption of permanent teeth is completed, reduction of alveolar bone and final forming of the pulp chamber for subsequent preparation for definitive dental restoration [3]. However, lack of care for denture by a child and its wear and plaque accumulation are the greatest limitations of this treatment [4]. Metal ceramic bridges in these cases have also to be postponed until marginal gingiva stability is established. Also, rigid fixation between two teeth would lead to difficult bone growth between them. Maryland bridges can be used as a replacement of missing teeth, but they are criticized for the lack of aesthetics caused by the presence of metal base and weak bonding between metal extensions and enamel [5, 6, 7].

Dental implants, on the other hand, are not recommended until the end of the growth period; due to ankylosis implant-bone bond that does not allow their growth together with facial bones presenting danger of their apical dislocation [8, 9]. Minimally invasive therapy is good choice in these cases. Adhesive bridges are an economical and simple solution that gives good aesthetic results in a single visit in order to preserve space for future implant placement. Fiber reinforced composites (FRC) are resin based materials that contain fibers to improve their physical characteristics. They were first mentioned in the literature in 1960s when Glass fibers were used for reinforcement of polymethyl methacrylate [10]. Different types of fibers, such as glass, carbon, kevlar and polyethylene fibers were added to composite materials in order to improve their properties [11]. Our study described the use of FRC technology in the production of adhesive bridges in a patient with hypodontia of permanent upper lateral incisors.

The aim of this study was to show one treatment option for hypodontia of permanent upper lateral incisors by creating adhesive composite bridge in one visit.

## CASE REPORT

A 15 years old female patient was referred to the Department of Prosthodontics, School of Dental Medicine, University of Belgrade, for the treatment of hypodontia of



**Figure 1.** Dental status of the patient at the beginning of treatment (hypodontia of upper permanent lateral incisors)

**Slika 1.** Izgled pacijenta na početku (hipodoncija gornjih stalnih lateralnih sekutića)



**Figure 2.** Partial acrylic denture

**Slika 2.** Parcijalna akrilatna proteza („žabica“) *in situ*



**Figure 3.** Study model with completed diagnostic modeling in wax

**Slika 3.** Model za studije sa završenim dijagnostičkim modelovanjem u vosku

permanent upper lateral incisors (Figure 1). During the past two years she wore a fixed orthodontic appliance, and at the end of orthodontic treatment at the age of 14 she was rehabilitated with partial denture as a temporary solution. Partial denture was not satisfactory treatment aesthetically or functionally causing the patient emotional insecurity (Figure 2).

After clinical examination and analysis of panoramic x-ray, impressions were taken for the study models. Their analysis in an articulator showed sufficient interocclusal space between the upper and lower anterior teeth to create fiber-reinforced adhesive bridge without the need for



**Figure 4.** Surface preparation, enamel etching with phosphoric acid

**Slika 4.** Priprema površine gleđi nagrizanjem ortofosfornom kiselinom



**Figure 5.** Application of special adhesive to enamel before adaptation of fibers

**Slika 5.** Nanošenje specijalnog adheziva za gleđ pre adaptacije vlakana



**Figure 6.** Base body modeling of the bridge from fibers and their placing on the teeth.

**Slika 6.** Oblikovanje osnove tela mosta od vlakana i njihovo postavljanje na zube

preparation of abutment teeth. Diagnostic modeling of upper lateral incisors was done so that the patient would have insight into the future restoration and a silicone key was designed for modeling missing incisors on the basis of test modeling in wax (Figure 3). In one visit, the adhesive bridge was made using a GC everStick C & B Fiber (GC, Europe) and a set of GC Gradia composite materials (GC, Europe) with the aim of temporary restoration of missing teeth. EverStick C & B fiber is made of over 4000 E salinized glass-fiber surrounded by bis-GMA and polymethyl methacrylate (PMMA). The required length of the fibers was measured on the study model. The fibers



**Figure 7.** Appearance of the adhesive fiber bridge after light curing  
**Slika 7.** Izgled tela adhezivnog mosta od vlakna nakon svetlosne polimerizacije



**Figure 8.** Modeling of the lateral incisor by composite layering technique  
**Slika 8.** Modelovanje lateralnog sekutića tehnikom slojevitog na-nošenja kompozita

were the length of interproximal space and a few mm longer on palatal surfaces of the abutment teeth. Enamel on palatal and proximal part of abutment teeth was etched with 37% phosphoric acid, rinsed with water, air dried and a thin layer of universal self etch adhesive G-aenial bond (GC, Europe) was applied (Figures 4,5). After curing a thin layer of flowable composite GC G-aenial Universal Flo (GC, Europe) was applied on prepared tooth surface. The fibers were placed using a special StickStepper instrument (GC, Europe) and then connector on one abutment tooth was light cured for 5-10 sec, while protecting with the instrument premature polymerization of the entire adapted fiber (Figures 6, 7, 8). Then after polymerization was carried out on the other connector. Gradual application and polymerization of the liquid composite layers formed the basis of the future adhesive bridge. The rest of the bridge was modeled using GC Gradia Direct composite (GC, Europe). The same procedure was repeated for the second adhesive bridge. The occlusion was checked and the restorations were polished (Figures 9, 10).

On the follow-up examination after two months, there was no observed damage of the bridges and a good oral hygiene was established. Patient was very pleased with the appearance and function of adhesive bridges.

## DISCUSSION

Hypodontia of lateral incisors in young patients is difficult to treat and certainly conventional 3 unit bridges cannot be done [12]. On the other hand, even after the completion of development and growth bridges are inferior therapeu-



**Figure 9.** The appearance of the completed right adhesive bridge  
**Slika 9.** Izgled završenog desnog adhezivnog mosta



**Figure 10.** Final appearance of the patient after both adhesive bridges completion  
**Slika 10.** Finalni izgled pacijenta po završetku oba adhezivna mosta

tic solution, because they reduce healthy tooth substance of the two supporting teeth and there is always danger of exposing the pulp during preparation [13, 7, 14].

Another treatment option is Maryland Bridge with a preparation on the palatal surface of abutment teeth for rest placement. However, the biggest disadvantage of these bridges is the necessity for the preparation of healthy teeth and inadequate aesthetics of metal base [7]. With the advent of modern technology in dentistry, porcelain Maryland bridges are replacing metal base but they cost more. Partial denture is one treatment option but it often breaks and can cause irritation of palatal mucosa when used for a longer period of time [15]. Implants are an appropriate solution for the replacement of lateral incisors in the case of hypodontia, but patients consider it expensive and invasive treatment, and their fear and anxiety for surgical procedures must not be ignored [7, 13, 15, 16].

In the clinical case we presented, as well as in other similar studies, age of the patient was clear contraindication for implant placement [12]. For all these reasons it was decided to do minimally invasive, conservative solution, with the use of fiber reinforced composite resin until the definitive prosthetic-implant therapy.

In the previous years, the development of fiber-reinforced composites (FRC) provided an opportunity for dentists to produce adhesive, esthetic bridges even in

one visit. FRC bridges are considered today an innovative solution and alternative to conventional fixed dental restorations and even implants in certain situations. In comparison with other prosthetic solutions, FRC bridges are quickly and easily made and they generally cost less [7]. Thin filaments incorporated into the base resin provide excellent resistance to fracture, fatigue, improved resistance to bending and tensile strength. The most common cause of failure is connector-cracking, delamination of the composite or fiber exposure [15].

Studies by other authors have shown the mean survival time of these bridges between 3–5 years, which makes them an appropriate transitional solution for the replacement of permanent anterior teeth in children. However, their disadvantages are reflected in the form of difficulty in maintaining oral hygiene as well as the inability to resist stronger occlusal forces [17, 18].

Within the limits of our study, we can say that adhesive bridges are quick, simple and clinically acceptable interim solution for the replacement of missing anterior teeth, but further clinical studies are required to verify their quality and efficiency.

## CONCLUSION

The use of fiber-reinforced composites offers a unique and effective possibility for replacing missing teeth. This technology enables production of metal-free, cost-effective, minimally invasive restoration that preserves tooth structure. These bridges are considered temporary therapeutic solution before finalization of conventional fixed restorations or implant therapy.

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Received: 07.06.2016 • Accepted: 18.08.2016

# Vlaknima ojačan kompozitni most kao zamena za neiznikli gornji stalni lateralni sekutić – prikaz bolesnika

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## KRATAK SADRŽAJ

Hipodoncija gornjih lateralnih sekutića predstavlja značajan estetski problem mladih osoba. Idealno rešenje ovog problema bi predstavljala ugradnja implantata i izrada krunice, ali je prisutan problem nezavršenog rasta i razvoja. Zbog toga je neophodno izraditi privremeno rešenje, kako bi se normalno odvijale funkcije stomatognatnog sistema i rešili estetski problemi, sve do izrade trajne nadoknade.

Cilj ovog rada bio je da se prikaže mogućnost zbrinjavanja hipodoncije stalnih gornjih lateralnih sekutića izradom adhezivnog mosta u jednoj poseti.

Pacijentkinja starosti 15 godina javila se na Kliniku za stomatološku protetiku Stomatološkog fakulteta u Beogradu sa hipodoncijom stalnih gornjih lateralnih sekutića. U anamnezi je navela da je nosila fiksni ortodontski aparat do svoje 14. godine, a potom bila rehabilitovana parcijalnom pločastom protezom, kojom nije bila zadovoljna ni estetski ni funkcionalno, što je značajno uticalo na njenu emocionalnu nesigurnost. Izrađen je adhezivni most primenom GC kompozitnih vlakana i setom GC kompozitnih materijala sa ciljem privremene nadoknade neizniklih zuba.

Primenjenom terapijom je postignut zadovoljavajući funkcionalni i estetski rezultat do momenta realizacije trajnog protetskog rešenja. Sačuvan je prostor za ugradnju dva endosealna implantata, a period adaptacije je pozitvno uticao na socijalni razvoj osobe u osetljivom pubertetskom dobu.

**Ključne reči:** adhezivni mostovi; hipodoncija lateralnih sekutića; vlaknima ojačani kompoziti

## UVOD

Hipodoncija ili nedostatak stalnih gornjih lateralnih sekutića uzrokovana je izostankom formiranja Zubnih klica u vilici i javlja se u 20% populacije i to nešto češće u stalnoj nego u mlečnoj denticiji [1, 2]. Pored funkcionalnog, hipodoncija predstavlja i značajan estetski problem mladim osobama u osetljivom periodu života. Rešavanje ovog problema nadoknadama visoke estetske vrednosti je uglavnom limitirano nezavršenim rastom i razvojem. Zamena prednjih zuba koji nedostaju, naročito kod dece, predstavlja veliki izazov za kliničare. Zato je multidisciplinarni pristup uz saradnju dečjeg stomatologa, ortodonta, protetičara i oralnog hirurga od izuzetnog značaja kako bi se očuvali prostori i noseća tkiva do definitivne terapije. Parcijalne proteze su često terapija izbora kod dece dok ne niknu stalni zubi (ne smanje se promene dimenzija alveolarne kosti i ne formiraju se komore pulpe) za kasniju izradu definitivne Zubne nadoknade [3]. Ali, nedostatak brige o protezi od strane deteta i njeno habanje i akumulacija plaka predstavljaju najveća ograničenja ove terapije [4]. Metalokeramički mostovi se u ovim slučajevima isto odlažu do trenutka dok se ne uspostavi stabilnost marginalne gingive, a takođe kruta fiksacija između dva zuba dovela bi do ometanog rasta između njih. Maryland mostovi se takođe mogu koristiti kao zamena zuba koji nedostaju, ali im se zamera nedovoljna estetika usled prisustva metalne osnove i slabe veze između metalnih produžetaka i gledi [5–7].

Dentalni implantati se, sa druge strane, ne preporučuju do završetka perioda rasta, zbog samog tipa veze implantata i kosti, jer za razliku od ankilotičnih zuba, oni ne prate rast kostiju lica i uvek je prisutna opasnost od njihove apikalne dislokacije [8, 9].

Minimalno invazivna terapija stvara mnogo veći izbor u daljem odabiru definitivne terapije. Metoda izbora je izrada adhezivnih mostova u cilju nadoknade neizniklih zuba i čini ekonomično i jednostavno rešenje koje daje dobre estetske rezultate u samo jednoj seansi.

Vlaknima ojačani kompoziti (FRC) materijali su na bazi smole koji sadrže vlakna koja poboljšavaju njihove fizičke karakteristike. Prvi put se u literaturi pominju 1960-ih, kada su staklena vlakna korišćena za ojačanje polimetil metakrilata [10].

Različite vrste vlakna, kao što su staklena, ugljenična, kevlar i polietilenска vlakna, dodavana su kompozitnim materijalima kako bi se unapredile njihove osobine [11].

U radu je opisana primena FRC tehnologije u izradi adhezivnih mostova kod hipodoncije stalnih gornjih lateralnih sekutića.

Cilj ovog rada bio je da se prikaže mogućnost zbrinjavanja hipodoncije stalnih gornjih lateralnih sekutića izradom adhezivnog mosta u jednoj poseti.

## PRIKAZ PACIJENTA

Pacijentkinja starosti 15 godina se javila na Kliniku za stomatološku protetiku Stomatološkog fakulteta Univerziteta u Beogradu sa hipodoncijom stalnih gornjih lateralnih sekutića (Slika 1). Prethodne dve godine nosila je fiksni ortodontski aparat, a po završetku ortodontske terapije od svoje 14. godine bila je rehabilitovana parcijalnom pločastom protezom kao privremenim rešenjem. Parcijalna pločasta proteza nije joj više odgovarala u estetskom, a ni u funkcionalnom smislu, što je dovelo do njene emocionalne nesigurnosti (Slika 2). Nakon kliničkog pregleda i analize ortopantomografskog snimka uzeti su otisci za izradu modela za studije. Njihovom analizom u artikulatoru utvrđeno je da postoji dovoljno interokluzalnog prostora između gornjih i donjih prednjih zuba za izradu adhezivnog mosta retiniranog staklenim vlaknima bez potrebe za preparaciju ležišta. Na modelima za studije urađeno je i dijagnostičko modelovanje u vosku gornjih lateralnih sekutića, kako bi pacijentkinja imala uvid u izgled buduće nadoknade, i napravljen je silikonski ključ za oblikovanje sekutića koji su nedostajali na osnovu probnog

modelovanja u vosku (Slika 3). Potom je, u jednoj poseti, direktnom metodom izrađen adhezivni most primenom GC Everstick C&B fiber vlakana (GC, Europe) i setom GC Gradia kompozitnih materijala (GC, Europe) sa ciljem privremene nadoknade neizniklih zuba. Everstick C&B vlakna napravljena su od preko 4000 E-staklastih silaniziranih vlakana okružena sa bis-GMA i polimetil metakrilatom (PMMA).

Potrebna dužina vlakana izmerena je na studijskom modelu. Vlakna su bila dužine interproksimalnog prostora sa prelaskom od nekoliko milimetara na palatalne površine zuba nosača. Palatalna i deo aproksimalne površine gledi zuba nosača pripremljeni su rastvorom 37% ortofosforne kiseline, isprani vodom, posušeni vazduhom i nanet je jedan sloj univerzalnog samonagrizajućeg adheziva G-aenial bond (GC, Europe) (Slike 4 i 5). Nakon polimerizacije adheziva nanet je tanak sloj tečnog kompozita GC G-aenial Universal Flo (GC, Europe) na pripremljene površine zuba. Postavljen je vlakno pomoću specijalnog Stick Stepper instrumenta (GC, Europe), a zatim izvršena svetlosna polimerizacija tokom 5–10 s spojnica na jednom zubu nosaču, istovremeno štiteći pomoću instrumenta da ne dođe do prerane polimerizacije celog adaptiranog vlakna (Slike 6, 7 i 8). Zatim je izvršena polimerizacija druge spojnice. Postepenim nanošenjem i polimerizacijom slojeva tečnog kompozita formirana je osnova budućeg adhezivnog mosta. Ostatak mosta izmodelovan je korišćenjem GC Gradia direct kompozita (GC, Europe). Isti postupak je ponovljen i pri modelovanju drugog adhezivnog mosta. Izvršena je provera okluzije i nadoknade su ispolirane (Slike 9 i 10).

Na kontrolnom pregledu posle dva meseca nisu uočena bilo kakva oštećenja mostova i utvrđena je dobra oralna higijena. Pacijentkinja je bila veoma zadovoljna izgledom i funkcijom adhezivnih mostova.

## DISKUSIJA

Hipodoncija lateralnih sekutića se kod mladih pacijenata ne može rešiti konvencionalnim mostovima od tri člana [12]. Sa druge strane, oni kao i definitivna nadoknada po završenom rastu i razvoju predstavljaju inferiorno terapijsko rešenje, jer se njima redukuje zdrava Zubna supstanca dva noseća zuba i uvek postoji opasnost od eksponiranja pulpe prilikom brušenja [13, 7, 14].

Druga terapijska mogućnost su Maryland mostovi sa preparacijom ležišta za naslove na palatalnoj strani zuba nosača. Međutim, kao najveći nedostaci ističe se neophodnost za preparacije ležišta na zdravim zubima, kao i neadekvatna estetika metalne osnove [7]. Sa napretkom savremenih tehnologija u

stomatologiji metalnu osnovu polako zamenjuju keramički Maryland mostovi, koji su znatno skuplji od konvencionalnih. Parcijalne pločaste proteze kao privremeno rešenje su sklone lomu i mogu dovesti do iritacije palatalne sluzokože kada se koriste duži vremenski period [15]. Implantati predstavljaju odgovarajuće rešenje za zamenu lateralnih sekutića u slučaju hipodoncije, ali ih pacijenti smatraju skupim i invazivnim tretmanom, a ne sme se zanemariti ni strah i anksioznost pacijenata zbog hirurške procedure [15, 16, 13, 7]. U kliničkom slučaju koji smo prezentovali, kao i u drugim sličnim studijama, godište pacijenta je bilo jasna kontraindikacija za njihovu ugradnju [12]. Zbog svega toga je odlučeno da se do definitivne protetsko-implantološke terapije primeni minimalno invazivno, konzervativno rešenje, uz upotrebu vlaknima ojačanih kompozitnih smola. Tokom prethodnih godina, razvoj vlaknima ojačanih kompozita (FRC) pružio je mogućnost stomatolozima da izrađuju adhezivne, estetske bezmetalne mostove čak u jednoj poseti. FRC mostovi smatraju se danas inovativnim rešenjem i alternativom konvencionalnim fiksним zubnim nadoknadama, pa čak i implantatima u nekim situacijama. U poređenju sa drugim protetskim rešenjima, FRC mostovi se jednostavno i brzo izrađuju i generalno manje koštaju [7]. Tanki filamenti inkorporirani u osnovnu smolu omogućavaju odličnu otpornost na lom, zamor, poboljšanu otpornost na savijanje i zateznu čvrstoću. Najčešći uzrok neuspeha je pucanje spojnica, delaminacija kompozita ili eksponiranje vlakana [15].

Studije nekih autora su pokazale da je srednje vreme opstajanja ovih mostova od tri do pet godina, što ih čini odgovarajućim prelaznim rešenjem za zamenu stalnih prednjih zuba kod dece. Ipak, njihovi nedostaci se ogledaju u vidu teškoća u održavanju oralne higijene, kao i nemogućnosti da se odupru jačim zagrijajnim silama [17, 18].

U okviru ograničenja jednog prikaza slučaja iz prakse, može se reći da adhezivni mostovi predstavljaju brzo, jednostavno i klinički prihvatljivo, prelazno rešenje za zamenu prednjih stalnih zuba koji nedostaju, ali su potrebne dalje kliničke studije kako bi se potvrdio njihov kvalitet i efikasnost.

## ZAKLJUČAK

Upotreba vlaknima ojačanih kompozita nudi jedinstvenu i efikasnu mogućnost zamene zuba koji nedostaju. Ova tehnologija omogućava izradu bezmetalne, ekonomične, minimalno invazivne nadoknade sa malim utroškom vremena i očuvanjem zubne supstance. Ovi mostovi se smatraju privremenim terapijskim rešenjem pre konačne izrade konvencionalnih fiksnih zubnih nadoknada ili implantološke terapije.

# Peripheral giant cell granuloma – case report

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## SUMMARY

Peripheral giant cell granuloma (PGCG) or "Epulis gigantocellularis" is the most common oral lesion that originates from giant cells. It typically manifests in the form of soft tissue tumor purple-red or red-watery color consisting of multinuclear giant cells in the mononuclear stroma and extravascular erythrocytes. This lesion is not considered true neoplasm, rather reactive lesion stimulated by local irritation and trauma. However, the cause is not known with certainty.

This paper presents a 13-year-old boy with a large lesion in the region of right maxillary canine that was retained in jaw despite favorable vertical position and available space to accommodate in the dental arch. The lesion was completely removed under local anesthesia and histopathological findings confirmed the diagnosis of PGCG. Postoperative period went without complications. After four months additional surgical procedure was needed due to the recurrence, which after tooth naturally erupted and positioned in the dental arch.

**Keywords:** Peripheral giant cell granuloma; Epulis gigantocellularis

## INTRODUCTION

Epulis name originates from the Greek epi – on and Oulon - gums, and, in general, considers any growth on gingiva regardless of etiology [1]. Clinically, most epulis lesions look similar and it is difficult to differentiate these from other tumor-like masses. Since large number of gingival lesions macroscopically looks similar to epulis, the literature has not yet determined the official classification of such lesions.

The most common epulis located on gingiva is made up of connective tissue and covered with stratified squamous epithelium - epulis gigantocellularis. It can be found in the literature under different names such as: peripheral giant cell tumor or peripheral giant cell granuloma. It is most commonly found in the intercanine sector on interdental papilla [2], more often on buccal side in the lower jaw [3]. It is five times more common than central giant cell granuloma that develops in bone [4].

Peripheral giant cell granuloma (PGCG) is of unknown etiology and has slow growth [2]. According to most authors, the cause may be permanent irritation or trauma [5]. In some patients diagnosed with PGCG cytomegalovirus has been isolated [6]. However, it has not been confirmed whether it is the cause or just contributes to the development of the disease.

Initially, mucosa shows unchanged color and looks thickened but PGCG eventually grows to an impressive mass, red or dark red in color and soft consistency that can destroy bone [2]. Also, ulcerations may be present on the surface. PGCG usually does not exceed the size of 5 cm in diameter, but can be bigger. Due to its volume it can

push away adjacent teeth [4]. Its base is narrower than its entire volume and impressions of adjacent structures of the oral cavity (teeth, cheeks, language) can be seen on its surface.

In the early stage of its development x-ray does not show any change however after some time changes become noticeable. At this point differential diagnosis should include the central giant cell granuloma.

Treatment is surgical and sometimes requires removing the tooth from the affected area. Recurrent lesions are quite common but they are usually interpreted as the result of inadequate surgery.

The aim of this case report was to present PGCG in 13-year-old boy that was removed by simple surgical procedure that enabled normal eruption of canine and its placement in the dental arch.

## CASE REPORT

A 13 -year-old boy presented at the Clinic for Oral Surgery, Faculty of Medicine Foca, with evident tumefaction on the alveolar ridge of maxilla in the region of the right canine. Lesion was 3.5×2.5cm in size, kidney-shaped, red-purple in color and localized in the space of mesial surface of the first right premolar up to the mid part of upper right central incisor, both on vestibular and palatal sides, fulfilling almost the entire vestibule (Figures 1 and 2). X ray revealed right canine present in the bone that could not erupt due to this lesion on gingiva (Figure 3). The patient underwent fine aspiration biopsy, which after histopathologic examination confirmed the presence of mul-



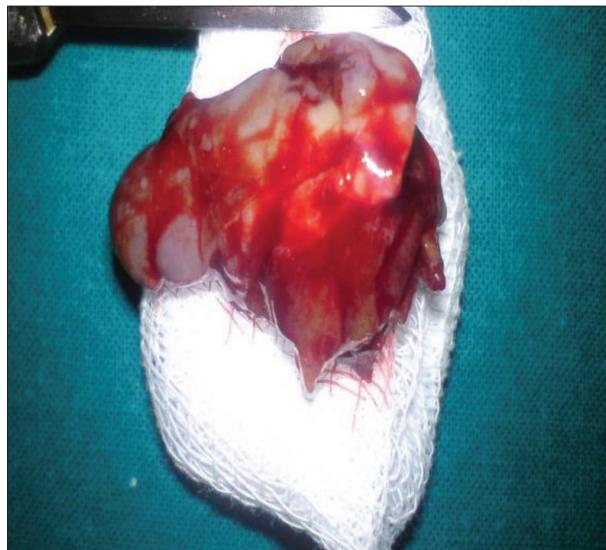
**Figure 1.** Epulis gigantocellularis – buccal view  
**Slika 1.** Epulis gigantocellularis – izgled sa vestibularne strane



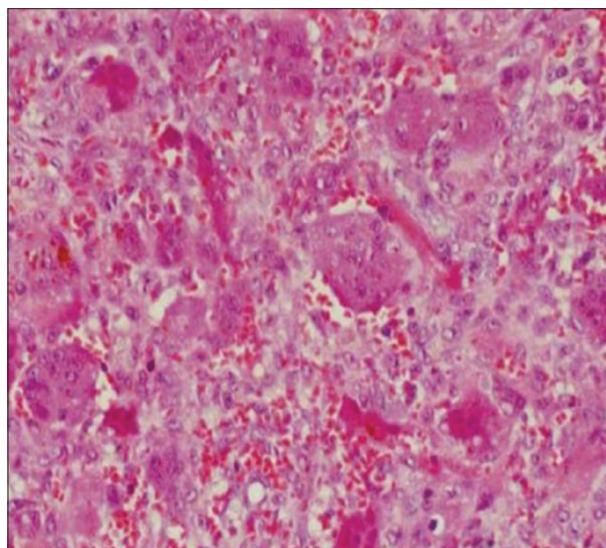
**Figure 2.** Epulis gigantocellularis- occlusal view  
**Slika 2.** Epulis gigantocellularis – izgled sa okluzalne strane



**Figure 3.** X-ray of unerupted upper right canine  
**Slika 3.** Rtg snimak neizniklog desnog gornjeg očnjaka



**Figure 4.** Epulis gigantocellularis completely removed  
**Slika 4.** Epulis gigantocellularis uklonjen u potpunosti



**Figure 5.** Histopathological finding of epulis gigantocellularis  
**Slika 5.** Histopatološki nalaz epulis gigantocellularisa

tinucleate giant cells with hemorrhage around. Spindle / inflammatory cells were not detected.

Surgical removal of the lesion was performed under local anesthesia when it was completely removed in one piece and sent for histopathological analysis (Figure 4). Histopathology revealed the presence of numerous multi-nuclear cells of different shapes and sizes, containing 8-15 nuclei in fibroblast stroma. Expanded endothelial cells with extravasal erythrocytes were also detected. Moreover, the presence of several giant cells within the vascular space, as well as signs of ossification within the stroma was observed (Figure 5). Based on found histopathological changes it was evident that it is a peripheral giant cell lesion. After surgical procedure was done packaged bandage was placed in the defect, which was removed seven days after the procedure.

After four months, the patient came back with the recurrence of the lesion in the form of blue-purple nodule 5 mm in size (Figure 6), which was again completely re-



**Figure 6.** Recurrence of the lesion in the area where it was removed previously

**Slika 6.** Recidiv na mestu uklonjenog epulis gigantocellularisa

moved under local anesthesia (Figure 7). Canine finally erupted into its space. One year after the intervention, there was no sign of possible recurrence while soft-tissue structures around the teeth fully healed.

## DISCUSSION

Etiology and nature of PGCG remains undefined. In the past there have been several proposed hypotheses that tried to explain the nature of multinuclear giant cells. These include the hypothesis that they are remnants of osteoclasts that perform natural resorption of primary dentition or that have arisen in response to violations of the periosteum.

PGCG can occur any time in life, but the most common incidence is in the mixed dentition [4]. The lesions are more common in the lower jaw [3, 7] while its size can reach over 2 cm in diameter. Clinically it is similar to pyogenic granuloma, but PGCG typically have blue-purple color compared to red pyogenic granuloma. Recently, there was published case of PGCG associated with dental implants [8]. Although PGCG develops in the soft tissue, local bone resorption of the alveolar ridge can be observed as well. Sometimes it is difficult to assess whether the tumor mass is peripheral lesion or central giant cell granuloma (CGCG) that originate in bone tissue of the alveolar ridge and spreads into gingiva [9, 10, 11].

Histologically, PGCG consists of islands of multinuclear giant cells in the stroma filled with oval and spindle mesenchymal cells and extravasal erythrocytes. Giant cells can contain several to several tens of nuclei. Some of them are large vesicular nuclei, while others are small, pyknotic nuclei. The origin of giant cells is unknown. Ultrastructural and immunological studies [12-16] have shown that they, in fact, originate from osteoclasts [17]. Some researchers believe that they represent reactive component of the lesion, in fact, mononuclear cells from bone marrow that



**Figure 7.** Recurrent lesion removed and retained tooth spontaneously erupted into the dental arch

**Slika 7.** Uklonjen recidiv i retinirani zub spontano sputen u Zubni luk

mature through the bloodstream and their sequential extravasation in response to an unknown stimulus from the stroma. This concept is based on the results of recent studies using cell culture and transplantation [18, 19], where giant cells with short life were found and disappeared in culture, in comparison with active proliferation of stromal cells.

According to the findings of Willing et al. [20] it was revealed that stromal cells secrete cytokines and other factors of cell differentiation, including monocyte chemo attractant protein-1 (MCP1), osteoclasts differentiating factor (ODF), and macrophage-stimulating factor (M-CSF). MCP1 is essential for osteoclast differentiation, indicating that stromal cells stimulate migration of monocytes into the tumor tissue fusing in multinucleated giant cells that resemble osteoclasts.

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Received: 25.04.2016 • Accepted: 27.07.2016

# Periferni gigantocelularni granulom – prikaz bolesnika

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## KRATAK SADRŽAJ

Periferni gigantocelularni granulom (PGCG) ili tzv. epulis gigantocellularis je najčešća oralna lezija porekla džinovskih ćelija. Obično se manifestuje u formi mekotkivnog tumora purpurno crvene ili crveno-lividne boje, koji se sastoji od više jedarnih džinovskih ćelija u mono jedarnoj stromi i ekstravazovanih eritrocita. Ova lezija uglavnom ne predstavlja pravu neoplazmu, a po svojoj prirodi može biti reaktivna jer je stimulisan trauma i lokalna iritacija, dok je uzrok najčešće nepoznat.

Ovaj prikaz predstavlja 13-godišnjeg dečaka sa velikom lezijom u frontalnoj regiji gornje vilice, u području očnjaka sa desne strane, koji je zaostao u vilici i pored povoljne vertikalne pozicije i dovoljno raspoloživog prostora za smeštaj u Zubni niz. Lezija je u potpunosti uklonjena u lokalnoj anesteziji, a histopatološki nalaz je bio karakterističan za PGCG. Postoperativni tok je bio bez komplikacija, a početni recidiv nakon četiri meseca je u ponovnom zahvatu kompletno uklonjen, nakon čega više nije bilo recidiva, a Zub se spontano spustio i smestio u Zubni niz.

**Ključne reči:** periferni gigantocelularni granulom; epulis gigantocellularis

## UVOD

Naziv epulis nastao je od grčkog *epi* – na i *oulon* – desni, a podrazumeva, generalno, svaku izraslinu na desnim bez obzira na njenu etiologiju [1]. Klinički, većina epulisa izgleda slično, te problem predstavlja razlikovanje tih tumoru sličnih masa. Budući da veliki broj promena na gingivi, bar makroskopski, izgleda identično epulisu, u dostupnoj literaturi još uvek nije utvrđena zvanična podela ovakvih lezija.

Najčešće uočena formacija epulisa na desnim, građena od vezivnog tkiva i prekrivena pločastoslojevitim epitelom, jeste epulis gigantocellularis. U literaturi se sreću sinonimi za ovu leziju kao što su: periferni gigantocelularni tumor ili periferni gigantocelularni granulom. Najčešće se javlja u interkaninom sektoru na interdentalnoj papili [2], češće na donjoj vilici sa vestibularne strane [3]. Pet puta je češći od centralnog gigantocelularnog granuloma, koji se razvija u kosti [4].

Periferni gigantocelularni granulom (PGCG) nepoznate je etiologije i sporog rasta [2]. Prema navodima većine autora, uzroci mogu biti stalne iritacije ili traume regije desni na kojima se javlja epulis [5]. Kod nekih pacijenata kojima je dijagnostikovan PGCG izolovan je citomegalovirus [6], pri čemu nije utvrđeno da li je on uzročnik ili samo doprinosi razvoju same bolesti.

U početku, sluzokoža je nepromenjene boje i zadebljala, da bi PGCG vremenom narastao kao impresivna tumefakcija crvene ili tamnocrvene boje i meke konzistencije, koja može da razara koštanu masu [2]. Na površini ove promene mogu se razviti ulceracije. Veličina PGCG obično ne prelazi dimenzije od 5 cm u prečniku, ali mogu biti i veći. Zbog svoje mase mogu razdvojiti susedne zube [4]. Za njega je karakteristično da mu je baza uža od celokupnog obima tumefekta, kao i da se na njemu mogu uočiti impresije susednih struktura usne šupljine (zubi, obrazi, jezik).

U početku razvoja bolesti na rendgenskom snimku se ne uočava rasvetljenje kosti, ali nakon određenog vremena te promene su i rendgenski vidljive. Tada diferencijalno dijagnostički može podsećati na centralni gigantocelularni granulom.

Terapija je isključivo hirurška i, ako je neophodno, mogu se i ukloniti zubi iz regije granuloma. Recidivi lezije su dosta česti, a najčešće se tumače kao posledica neadekvatnog hirurškog zahvata.

Cilj ovog rada je bio da se predstavi neuobičajena lokalizacija perifernog gigantocelularnog granuloma i ukaže na jednostavno hirurško rešenje kojim je istovremeno omogućeno nicanje neizniklog očnjaka i njegovo smeštanje u Zubni niz.

## PRIKAZ BOLESNIKA

Na Kliniku za oralnu hirurgiju Medicinskog fakulteta u Foči roditelji su doveli 13-godišnjeg dečaka sa evidentnim tumeftatom na alveolarnom grebenu gornje vilice u regiji očnjaka sa desne strane. Lezija veličine  $3,5 \times 2,5$  cm, bubrežastog oblika, crveno-ljubičaste boje bila je lokalizovana od mezijalne strane premolara do polovine prvog gornjeg sekutića (i sa vestibularne i sa palatalne strane) i ispunjavala je skoro ceo vestibulum (Slike 1 i 2). Nakon urađenog retroalveolarnog rendgen snimka (Slika 3) uočeno je i prisustvo neizniklog očnjaka u vilici. Urađena je fina aspiraciona biopsija, koja je nakon histopatološkog pregleda potvrdila prisustvo više jedarnih džinovskih ćelija sa hemoragijom u okolini. Vretenaste/upalne ćelije nisu detektovane.

Lezija je u lokalnoj anesteziji u potpunosti uklonjena od okoline u jednom komadu (Slika 4) i poslata na histopatološku analizu. Histopatološki je uočeno prisustvo brojnih više jedarnih ćelija različitog oblika i veličine, koje su sadržavale od 8 do 15 jedara u fibroblastnoj stromi. Uočene su i proširene endotelne ćelije sa ekstravazovanim eritrocitim. Takođe je uočeno i prisustvo nekoliko džinovskih ćelija unutar vaskularnih prostora, kao i znaci osifikacije unutar same strome (Slika 5). Na osnovu uočenih histopatoloških promena evidentno je bilo da se radi o perifernoj gigantocelularnoj leziji. Defekt nastao nakon uklanjanja pakovan je hirurškim zavojem, koji je uklonjen sedam dana posle intervencije.

Nakon četiri meseca pacijent se javio sa recidivom u formi plavo-ljubičastog čvorića veličine pet milimetara (Slika 6), koji je ponovo u lokalnoj anesteziji potpuno uklonjen i pakovan hirurškim zavojem (Slika 7). Očnjak je zauzeo svoje mesto u Zubnom nizu. Godinu dana nakon intervencije nije bilo nikakvih znakova eventualnog recidiva, a mekotkivne strukture u okolini zuba u potpunosti su zarasle.

## DISKUSIJA

Etiologija i priroda PGCG i dalje je nedefinisana. U prošlosti su postojale hipoteze koje su pokušale da objasne prirodu više jedarnih džinovskih ćelija, uključujući i hipoteze da su one ostaci osteoklasta, koji vrše prirodnu resorpciju mlečne dentitije ili da su nastale kao reakcija na povrede periosta.

PGCG se može javiti tokom celog života, ali sa najčešćom incidencijom u mešovitoj dentitiji [4]. Lezije su češće u donjoj vilici [3, 7], a u svojoj veličini mogu dosezati i preko dva centimetra u promeru. Klinički je sličan piogenom granulomu, ali se razlikuje uglavnom po plavo-ljubičastoj boji u poređenju sa crvenom bojom tipičnog piogenog granuloma. Nedavno je i objavljen slučaj PGCG povezanog sa dentalnim implantima [8]. Iako se PGCG razvija u mekom tkivu, često se zapoža i lokalna resorpacija kosti alveolarnog grebena. S vremena na vreme teško je oceniti da li je tumorska masa periferna lezija ili je centralni gigantocelularni granulom (CGCG) nastao u koštanom tkivu alveolarnog grebena, koji se širi u meka tkiva gingive [9, 10, 11].

Histološki, PGCG se sastoji od ostrva više jedarnih džinovskih ćelija u stromi ispunjenoj ovalnim i vretenastim mezenhimal-

nim ćelijama i ekstravazovanim eritrocitima. Džinovske ćelije mogu sadržavati nekoliko jedara pa do nekoliko desetina. Neka od njih su velika vezikularna jedra, dok su kod drugih prisutna mala, piknotična jedra. Poreklo džinovskih ćelija je nepoznato. Ultrastrukturne i imunološke studije [12–16] pokazale su da su one, zapravo, nastale od osteoklasta [17]. Većina smatra da one predstavljaju reakcionu komponentu lezije, i da su to mononuklearne ćelije iz koštane srži koje su tu dospele putem krvotoka i njihovom sledstvenom ekstravazacijom, a predstavljaju odgovor na podsticaj iz same strome. Ovaj koncept se zasniva na rezultatima nedavnih studija, korišćenjem kulture ćelija i transplantacijom [18, 19], gde su pronađene džinovske ćelije, kratkog životnog veka, koje nestaju u kulturi, u poređenju sa aktivnom proliferacijom stromalnih ćelija.

Prema nalazima Willing et al. [20] otkriveno je da stromalne ćelije luče citokine i druge faktore diferencijacije ćelija, uključujući monocitni hematotraktantni protein-1 (MCP1), osteoklastični diferencijski faktor (ODF) i makrofag stimulišući faktor (M-CSF). MCP1 je od suštinskog značaja za diferencijaciju osteoklasta i ukazuje da stromalne ćelije stimulišu migraciju monocita u tkivo tumora fuzionišući se u multijedarne džinovske ćelije, nalik osteoklastima.

## Dental links / Stomatološki linkovi

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**MEDLINE**

[http://www.nlm.nih.gov/databases/databases\\_medline.html](http://www.nlm.nih.gov/databases/databases_medline.html)

**OPERATIVE DENTISTRY**

<http://www.jopdent.org/journal/journal.php>

**JOURNAL OF CONTEMPORARY DENTAL PRACTICE**

<http://thejcdp.com/>

**JOURNAL OF THE AMERICAN DENTAL ASSOCIATION (JADA)**

<http://jada.ada.org/>

**BRITISH DENTAL JOURNAL**

<http://www.nature.com/bdj/index.html>

**JOURNAL OF DENTAL RESEARCH**

<http://www.iadr.com/>

**CANADIAN MEDICAL ASSOCIATION JOURNAL (CMAJ)**

<http://www.cmaj.ca/>

**JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION (JAMA)**

<http://jama.ama-assn.org/>

**JOURNAL OF THE CANADIAN DENTAL ASSOCIATION (JCDA)**

<http://www.cda-adc.ca/jcda/>

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<http://www.joponline.org/loi/jop>

**JOURNAL OF ORTHODONTICS**

<http://jorthod.maneyjournals.org/>

**INTERNATIONAL JOURNAL OF PAEDIATRIC DENTISTRY**

<http://www.wiley.com/bw/journal.asp?ref=0960-7439>

**AUSTRALIAN DENTAL JOURNAL**

<http://www.ada.org.au/Publications/adj.aspx>

## Da li ste pažljivo čitali radove?

1. Intenzitet pulpnog bola posle delovanja električnog i hladnog nadražaja je proveravan:
  - a) kod dece
  - b) kod odraslih žena
  - c) kod odraslih muškaraca
2. Koncentracija kojih elektrolita je određivana u nestimulisanoj pljuvački?
  - a) samo natrijuma
  - b) samo kalcijuma
  - c) natrijuma, kalijuma i kalcijuma
3. Hipodonciju je moguće rešiti:
  - a) samo implantima
  - b) samo ortodontski
  - c) izradom adhezivnog mosta
4. Rezultati ispitivanja uticaja navika u ishrani na pojavu karijesa su pokazali da je cirkularnim karijesom bilo zahvaćeno:
  - a) 34,9 % dece
  - b) 24,9% dece
  - c) 54,9% dece
5. Koncentracija natrijuma u nestimulisanoj pljuvački sakupljenoj epruvetama iznosila je:
  - a) 8,43 mmol/L
  - b) 7,90 mmol/L
  - c) 13,54 mmol/L
6. Adhezivni most obezbeđuje:
  - a) dobru funkciju i estetiku
  - b) samo funkciju
  - c) samo estetski momenat
7. Ph i puferski kapacitet pljuvačke je određivan kod:
  - a) zdravih ispitanika
  - b) obolelih ispitanika
  - c) i kod zdravih i kod obolelih ispitanika
8. Provera intenziteta pulpnog bola je urađena kod:
  - a) 20 zdravih žena
  - b) 60 zdravih žena
  - c) 80 zdravih žena
9. Uzorak dece za ispitivanje navika u ishrani obuhvatio je:
  - a) 192 deteta
  - b) 152 deteta
  - c) 132 deteta
10. Koncentracija elektrolita je određivana kod:
  - a) nestimulisane pljuvačke
  - b) stimulisane pljuvačke
  - c) nestimulisane i stimulisane pljuvačke
11. Slabija bolna osjetljivost pulpe je zabeležena:
  - a) kod žena u lutealnoj fazi
  - b) kod žena u menstrualnoj fazi
  - c) kod žena u menopauzi
12. Hipodoncija je podrazumevala nedostatak:
  - a) stalnih centralnih sekutića
  - b) stalnih očnjaka
  - c) stalnih lateralnih sekutića
13. Ph pljuvačke sakupljene saline je iznosila:
  - a) 7,05
  - b) 7,35
  - c) 5,31
14. Osetljivost pulpe je proveravana kod:
  - a) gornjeg centralnog sekutića
  - b) donjeg centralnog sekutića
  - c) donjeg prvog molara

15. Adhezivni most kao protetsko rešenje je izrađen:
  - a) od kompozita
  - b) od fiber vlakana i kompozita
  - c) od keramike
16. Koncentracija Na i K u pljuvački je određivana:
  - a) metodom plamene emisione fotometrije
  - b) metodom spektrometrije
  - c) titracijom
17. Pacijentkinja sa hipodoncijom je imala:
  - a) 15 godina
  - b) 25 godina
  - c) 35 godina
18. Intenzitet pulpnog bola je bio značajno manji:
  - a) kod žena u menopauzi
  - b) kod žena u reproduktivnoj dobi
  - c) kod žena u lutealnoj fazi
19. Uticaj navika u ishrani na pojavu karijesa je proveravan kod dece uzrasta:
  - a) do 12 meseci
  - b) do 24 meseca
  - c) do 36 meseci
20. Veći prag nadražaja je registrovan:
  - a) u lutealnoj fazi
  - b) u menstrualnoj fazi
  - c) kod žena u menopauzi

Odgovore slati na email adresu Uredništva časopisa „Stomatološki glasnik Srbije“. Tačni odgovori na pitanja će se vrednovati u skladu s Pravilnikom o kontinuiranoj medicinskoj edukaciji zdravstvenih radnika.

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Народна библиотека Србије, Београд

616.31

**STOMATOLOŠKI glasnik Srbije** = Serbian  
Dental Journal / главни и одговорни уредник  
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> - Тромесечно

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

Poznań, Poland

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