

ZNAČAJ FOSFATNOG PUFERA PLJUVAČKE U EVALUACIJI UČESTALOSTI KARIJESA KOD DECE

THE SIGNIFICANCE OF PHOSPHATE BUFFER IN THE EVALUATION OF CARIES INCIDENCE IN CHILDREN

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Abstrakt

Fosfatni pufer je dominantni pufer nestimulisanе pljuvačke. Pufer predstavlja kombinaciju primarnog i sekundarnog fosfata. Ovom prospektivnom studijom je obuhvaćeno 123 dece sa stalnom denticionom, približno jednake polne zastupljenosti. Koncentracija fosfata je određivana molibdenskom reakcijom po spektrofotometrijskoj metodi Goldenberg-a i Fernandez-a. U grupi ispitanika sa niskim karijes rizikom u obe vrste pljuvačke uočene su značajno više vrednosti fosfata nego u grupi sa visokim karijes rizikom. Veći kapacitet fosfatnog pufera u stimulisanoj pljuvački obe ispitivane grupe pacijenata, ukazuje na značajnu puferšku aktivnost fosfatnog pufera nestimulisanе pljuvačke. Nivo fosfatnog pufera pokazuje negativnu korelaciju sa incidencijom karijesa.

Cljučne reči: pljuvačka, fosfatni pufer, karijes

Abstract

Phosphate buffer is a predominant buffer in unstimulated saliva. The buffer is a combination of primary and secondary phosphate. This prospective study enrolled 123 children with permanent dentition, with a similar number of males and females. Phosphate concentrations were determined using the molybdenum reaction by Goldenberg and Fernandez spectrophotometry method. In the group of examinees with low caries risk in both stimulated and unstimulated saliva groups significantly higher phosphate values were observed compared to the high caries risk group. A higher capacity of phosphate buffer in stimulated saliva in both studied patient groups indicate significant buffer activity of phosphate buffer in unstimulated saliva. The level of phosphate buffer is negatively correlated with caries incidence.

Key words: saliva, Phosphate buffer, caries

Uvod

Fosfatni pufer je dominantni pufer nestimulisanе pljuvačke. Pufer predstavlja kombinaciju primarnog i sekundarnog fosfata ($H_2PO_4^{2-}$ i $-HPO_4^{3-}$), čija koncentracija u nestimulisanoj pljuvački iznosi 7-8 mM/L, dok se za vreme stimulisane salivacije ova vrednost smanjuje na 2-3 mM/L¹.

Svojom koncentracijom od 7-8 mM/L u nestimulisanoj pljuvački dovodi do snižavanja pH pljuvačke sve do 6,1, zbog čega ona postaje blago zakiseljena. Blaga kiselost povećava moć rastvaranja pljuvačke u uslovima kada je ukupna zapremina nestimulisanе pljuvačke 20 do 30 puta manja. Efekti samočišćenja u ovom slučaju neće biti 20 do 30 puta manji, već naprotiv veći². Porast koncentracije fosfata deluje na adsorbovane salivarne glikoproteine desorbujuće, tako da se formirana zubna pelikla razgrađuje i uklanja sa površine zuba, a zubi čiste od ovih naslaga. Zbog ove svoje funkcije fosfatni pufer je u pravom smislu reči čistač oralne sredine^{2,3}.

Introduction

Phosphate buffer is a predominant buffer in unstimulated saliva. The buffer is a combination of primary and secondary phosphate ($H_2PO_4^{2-}$ i $-HPO_4^{3-}$), the concentration of which is 7-8 mM/L in unstimulated saliva, while the value decreases to 2-3 mM/L during stimulated salivation¹.

With its concentration of 7-8 mM/L in unstimulated saliva the buffer decreases salivary pH to 6.1, making it mildly acidic. Mild acidity makes the saliva better solvent in the conditions when the total volume of unstimulated saliva is reduced 20 to 30 times. The effects of self-cleaning will not be reduced 20 to 30 times in this case, but just the opposite, the effects will be more pronounced². Higher phosphate concentrations act upon salivary glycoproteins in a desorbing way, so that formed dental pellicle is broken down and removed from teeth surface and teeth are being cleaned from these sediments. This function of phosphate buffer makes it a cleanser of oral environment^{2,3}.

Ipitanci i metode

O vom prospektivnom studijom je obuhvaćeno 123 dece sa stalnom denticijom, približno jednake polne zastupljenosti, uzrasta od 13 do 15 godina (stratifikovan uzorak) u Osnovnoj Školi «Dositej Obradović» u Nišu.

Razlog zbog čega su izabrana deca viših razreda osnovne škole, sa prisutnom stalnom denticijom, je želja da se izbegne mešovita denticija, jer je KEP (K = karijes, E = ekstrahovani, P = plombirani, što predstavlja ukupan broj zuba sa karijesom, zuba sa plombom i izvađenih zuba) u uslovima mešovite denticije u velikoj meri posledica karijesa mlečnih zuba, čime bi bila «maskirana» prava slika o KEP-u pacijenta, čime bi dobijeni rezultati bili kompromitovani.

Svakom pacijentu je obavljen sistematski stomatološki pregled zuba i određen KEP stalnih zuba. Pacijenti su zatim podeljeni prvo u dve grupe, na osnovu KEP-a svakog deteta.

Prva grupa ispitanika obuhvata 52 pacijenta čiji je prosečan KEP nula (0) - deca sa niskim rizikom za nastanak karijesa. U drugoj grupi se nalazi 71 pacijent čiji je prosečan KEP po detetu 7,7 - deca sa visokim rizikom za nastanak karijesa.

Uzimanje uzoraka pljuvačke za potrebe izrade doktorske disertacije odobreno je od strane Etičkog Komiteta Medicinskog fakulteta u Nišu Odlukom broj **01-7044** od 30.10.2007. godine.

Pacijentima iz obe grupe uzeta su po dva uzorka pljuvačke - nestimulisane i stimulisane. Uzorci pljuvačke su sakupljeni u sterilne epruvete. Uzorci nestimulisane pljuvačke su uzimani između obroka, skupljanjem pljuvačke trenutno prisutne u ustima, pre podne. Uzorci stimulisane pljuvačke su uzimani nakon što su pacijenti, takođe između obroka u jutarnjim časovima, žvakali medicinski parafin pet minuta, što je izazvalo obimnu sekreciju stimulisane pljuvačke. Ovim putem definisane su četiri grupe uzoraka pljuvačke (Tabela 1).

Tabela 1. Grupe uzoraka ispitivane pljuvačke

<i>Grupa uzoraka</i>	<i>Vrsta pljuvačke</i>	<i>Stepen rizika</i>	<i>Broj uzoraka</i>
I - NNR	Nestimulisana	Nizak	52
II - SNR	Stimulisana	Nizak	52
III - NVR	Nestimulisana	Visok	71
IV - SVR	Stimulisana	Visok	71

Examinees and methods

This prospective study enrolled 123 children with permanent dentition, with a similar number of males and females, aged 13 to 15 years (stratified sample) in „Dositej Obradović“ elementary school in Niš.

The reason why we chose the children with permanent dentition was the wish to avoid mixed dentition, since the DMF (D-decay; M-missing; F-field; making up the total number of caries-involved teeth, extracted teeth and those treated with fillings) in mixed dentition cases is primarily the consequence of milk teeth caries, „masking“ the real picture of DMF and compromising our results.

Each patient's teeth were systematically examined and DMF determined for permanent teeth. The patients were firstly divided into two groups based on the DMF of each child.

The first group of examinees included 52 patients with „0“ average DMF - the children with low caries risk. There were 71 patients in the second group with 7.7 average DMF - those with high risk of developing caries.

Salivary sampling for the purpose of doctoral dissertation was approved by the Ethics Committee of the Faculty of Medicine (decision number 01-7044 of October 30, 2007).

We took two samples of saliva from each child - unstimulated and stimulated saliva. The samples were collected in sterile vials. The samples of unstimulated saliva were taken in the morning, between meals, collecting the saliva present in the mouth at that moment. The samples of stimulated saliva were taken between meals in the morning too, after the children chewed medical paraffin for five minutes (which induced ample secretion of stimulated saliva). Four groups of saliva samples were thus defined (Table 1).

Table 1. Groups of saliva samples

<i>Sample group</i>	<i>Type of saliva</i>	<i>Risk degree</i>	<i>Number of samples</i>
I - NNR	Unstimulated	Low	52
II - SNR	Stimulated	Low	52
III - NVR	Unstimulated	High	71
IV - SVR	Stimulated	High	71

Koncentracija fosfata je određivana molibdenskom reakcijom po spektrofotometrijskoj metodi Goldenberg-a i Fernandez-a (1966), modifikovanoj od strane Bardow-a i sar. (2000). Pljuvački je dodavana reakciona smeša (10% TCA, 1% urea i 3% Morova so). Nakon 10 minuta vršeno je centrifugiranje i supernatantima su dodavani koncentrovana H_2SO_4 i 4,5% amonijum molibdat u dejonizovanoj vodi. Nakon 20 minuta apsorbancija je merena na 700 nm. Standardna kriva je pravljenjena u rasponu od 0-10 mmol/L fosfata.

Rezultati

Rezultati analize kvantitativnih vrednosti fosfata nestimulisane ($5,61 \pm 0,3$ mmol/L) i stimulisane pljuvačke ($8,68 \pm 2,2$ mmol/L) u grupi sa niskim karijes rizikom ukazuju na statistički veoma izraženi porast koncentracije nakon stimulacije ($p < 0,001$) (Grafikon 1).

Statistički značajna porast koncentracije fosfata nakon stimulacije uočena je analizom vrednosti u nestimulisanoj ($4,06 \pm 1,2$ mmol/L) i stimulisanoj pljuvački ($6,22 \pm 0,8$ mmol/L; $p < 0,001$) dece sa visokim rizikom za nastanak karijesa (Grafikon 2).

U grupi ispitanika sa niskim karijes rizikom uočavaju se statistički značajno više vrednosti fosfata, kako u nestimulisanoj ($p < 0,001$), tako i u stimulisanoj pljuvački ($p < 0,001$) u odnosu na ispitivanu grupu sa visokim karijes rizikom (Grafikon 3).

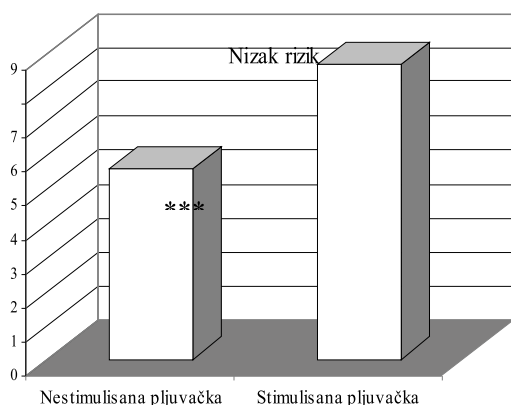
Phosphate concentrations were determined using the molybdenum reaction by Goldenberg and Fernandez spectrophotometry method (1966), modified by Bardow et al. (2000). Reaction mixture (10% TCA, 1% urea and 3% Mohr's salt) was added to saliva samples and after 10 minutes centrifugation was done and supernatants were added with concentrated H_2SO_4 and 4,5% ammonium molybdate in deionized water. Twenty minutes later the absorbance was measured at 700 nm. Standard curve was drawn in the phosphate range of 0-10 mmol/L.

Results

Results of the analysis of quantitative phosphate value in unstimulated ($5,61 \pm 0,3$ mmol/L) and stimulated saliva ($8,68 \pm 2,2$ mmol/L) in low caries risk group indicated a statistically significant rise in concentration after stimulation ($p < 0,001$) (Graph 1).

A statistically significant rise in phosphate concentration after stimulation was observed after the analysis of values in unstimulated ($4,06 \pm 1,2$ mmol/L) and stimulated saliva ($6,22 \pm 0,8$ mmol/L; $p < 0,001$) of the children with high caries risk (Graph 2).

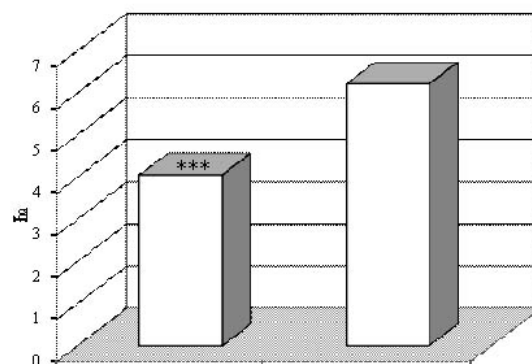
In the group of examinees with low caries risk we observed statistically significant higher phosphate values in both unstimulated ($p < 0,001$) and stimulated saliva ($p < 0,001$) compared to the examined group with high caries risk (Graph 3).



*** $p < 0,001$ vs stimulisana; *** $p < 0,001$ vs stimulated

Grafikon 1. Vrednosti koncentracije fosfata pre i nakon stimulacije u grupi dece sa niskim rizikom za nastanak karijesa

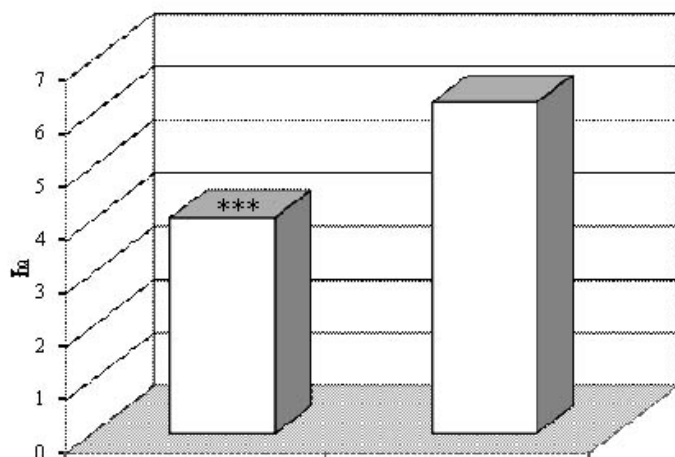
Graph 1. Values of phosphate concentration before and after stimulation in low caries risk group of children



*** $p < 0,001$ vs stimulisana; *** $p < 0,001$ vs stimulated

Grafikon 2. Vrednosti koncentracije fosfata pre i nakon stimulacije u grupi dece sa visokim rizikom za nastanak karijesa

Graph 2. Phosphate concentrations before and after stimulation in the group of children with high caries risk



*** $p < 0,001$ vs *stimulisana*;
 *** $p < 0,001$ vs *stimulated*

Grafikon 3. Vrednosti koncentracije fosfata pljuvačke u odnosu na karijes rizik grupu i vrstu pljuvačke

Graph 3. Salivary phosphate concentrations related to caries risk groups and induction of salivation

Diskusija

Fosfatni pufer, uz bikarbonatni pufer i proteine, predstavlja osnovu puferskog sistema pljuvačke. Fosfatni pufer je primarni pufer nestimulisane pljuvačke i kao takav izuzetno je važan u fiziologiji bazične, nestimulisane pljuvačke, koja se u ustima nalazi oko 22 sata tokom dana, u periodu kada nema lučenja stimulisane pljuvačke. Obzirom na tako dug period u kome nestimulisana pljuvačka deluje na zubna tkiva, kapacitet fosfatnog pufera igra značajnu ulogu u etiopatogenezi karijesa⁴.

Rezultati istraživanja pokazuju da su u grupi dece sa niskim karijes rizikom vrednosti fosfata u stimulisanoj pljuvački bile statistički značajno veće. Sličan trend pokazivali su i rezultati u ispitivanoj grupi dece sa visokim karijes rizikom. Dobijeni rezultati su u skladu sa istraživanjima drugih autora^{5,6}. Autori objašnjavaju da su vrednosti prikupljane nestimulisane pljuvačke u izvesnoj meri kompromitovane činjenicom da je fosfatni pufer već aktivan u usnoj duplji, i da se veći deo ukupne količine fosfata već potrošio neutrališući kiselost u ustima i održavajući pljuvačku u uslovima njene individualne fiziološke homeostaze⁴⁻⁶. Za razliku od rezultata nestimulisane pljuvačke, koja u sebi sadrži kvantitativno delimično istrošeni fosfatni pufer, prikupljanjem stimulisane pljuvačke dobija se puna, tek izlučena vrednost fosfata, koja je paradoksalno u trenutku merenja veća od vrednosti bazične pljuvačke⁷.

Discussion

Phosphate buffer, together with bicarbonate buffer and proteins, forms the the basis of salivary buffer system. It is the primary buffer of unstimulated saliva, very important in the physiology of basic, unstimulated saliva, present in the mouth for approximately 22 hours a day, in the period when stimulated saliva is absent. Since the period of time during which unstimulated saliva acts upon dental tissues is rather long, the capacity of phosphate buffer plays an important role in caries etiopathogenesis⁴.

The results of our study demonstrated significantly higher phosphate values in stimulated saliva in the group of children with low caries risk. The results obtained in the group of children with high caries risk demonstrated similar trends. These results corroborate other authors' investigations^{5,6}. The authors explained that the values of collected unstimulated saliva were compromised to a certain level by the fact that phosphate buffer was already active in the mouth cavity and that a larger portion of the total phosphate amount was spent to neutralize acidity in the mouth, preserving saliva in its individual physiologic homeostasis⁴⁻⁶. In contrast to the results with unstimulated saliva, which contains quantitatively partly depleted phosphate buffer, collecting of stimulated saliva provides us with full, just excreted phosphate value, paradoxically higher at the moment of measuring than the value for basic saliva⁷.

Bardow i saradnici⁷ ističu da je koncentracija neorganskih fosfata, poput koncentracije bikarbonata u celoj pljuvački, zavisna i od stepena lučenja. U fiziološkim uslovima, pri intenzitetu lučenja nestimulisane pljuvačke od 0.55 ml/min, sistem fosfatnog pufera predstavlja oko polovinu ukupnog puferskog kapaciteta između pH 5 i pH 7. Doprinos fosfatnog pufera u stimulisanoj pljuvački, čiji je prosečni intenzitet lučenja 1.66 ml/min, znatno je manji, zbog manje koncentracije fosfata⁷. Po Kreusser-u i saradnicima⁸, osnovna koncentracija HPO_4^{2-} jona raste sa povećanjem lučenja pljuvačke, praćenog povećanjem pH vrednosti pljuvačke.

Koncentracija fosfata u nestimulisanoj pljuvački viša je od one u stimulisanoj pljuvački. Nestimulisana pljuvačka sadrži oko 5 mmol/L ukupnih fosfata, dok stimulisana može sadržati i do ispod 3 mmol/L⁹. Zavisno od pH vrednosti pljuvačke, fosfati su zastupljeni u različitim jonskim formama, prema vrednostima njihove pK: $\text{H}_3\text{PO}_4 \leftrightarrow \text{H}_2\text{PO}_4^- + \text{H}^+$; $\text{H}_2\text{PO}_4^- \leftrightarrow \text{HPO}_4^{2-} + \text{H}^+$; $\text{HPO}_4^{2-} \leftrightarrow \text{PO}_4^{3-} + \text{H}^+$.

Pri fiziološkom pH nivou (oko 7), većina fosfata je prisutna u formi dihidrogenofosfata (H_2PO_4^-) i hidrogenofosfata (HPO_4^{2-}). H_2PO_4^- ima maksimalni puferski kapacitet na 7.21 pri 25^o C¹⁰.

Studija Bardow-a i saradnika¹¹ pokazuje da je pH vrednost i puferski sastav nestimulisane pljuvačke, sa dominantnim fosfatnim puferom, mnogo važniji činilac od istih parametara u stimulisanoj pljuvački u eksperimentu sa korenskom karijesnom lezijom *in situ*. Ovi rezultati su u saglasnosti sa prethodnim istraživanjima¹² efekata pljuvačke na nastanak karijesa, koji su takođe usmereni na parametre nestimulisane pljuvačke. Najznačajnija korelacija sa svim demineralizacionim činiocima je ustanovljena upravo sa koncentracijom ukupnih fosfata i proteina nestimulisane pljuvačke. Tako se može zaključiti da visok nivo salivarnih fosfata može imati potencijal da redukuje demineralizaciju aktivnih progresivnih karijesnih lezija¹¹. Dobljeni rezultati istraživanja u ovoj disertaciji u saglasnosti su sa literaturnim podacima, čime i ova naša studija doprinosi opšte prihvaćenom stavu da je vrednost fosfatnog pufera jedan od važnih parametara koji može uticati na incidencu karijesa.

Bardow et al.⁷ pointed out that the concentration of inorganic phosphates, similar to the bicarbonate concentration in whole saliva, was dependent too upon the intensity/degree of excretion. In physiologic conditions, at the excretion intensity level of 0.55 ml/min, the system of phosphate buffer represents about half of the total buffer capacity between pH 5 and pH 7. The contribution of phosphate buffer in stimulated saliva, the average intensity of secretion is 1.66 ml/min, is markedly smaller due to lower phosphate concentration⁷. After Kreusser et al.⁸, the basic HPO_4^{2-} ion concentration rises with increased excretion of saliva, associated with a rise in salivary pH value.

Phosphate concentration in unstimulated saliva is higher than those in stimulated saliva. Unstimulated saliva contains around 5 mmol/L of total phosphates, while stimulated saliva may contain as low as 3 mmol/L or less phosphates⁹. Depending on the salivary pH value, phosphates can be found in various ionic forms, according to their pK values: $\text{H}_3\text{PO}_4 \leftrightarrow \text{H}_2\text{PO}_4^- + \text{H}^+$; $\text{H}_2\text{PO}_4^- \leftrightarrow \text{HPO}_4^{2-} + \text{H}^+$; $\text{HPO}_4^{2-} \leftrightarrow \text{PO}_4^{3-} + \text{H}^+$.

At physiologic pH (around 7), most phosphates are present in the form of dihydrogen phosphate (H_2PO_4^-) and hydrogen phosphate (HPO_4^{2-}); H_2PO_4^- exerts its maximal buffer capacity at 7.21 and at 25^o C¹⁰.

The study of Bardow et al.¹¹ demonstrated that pH value and buffer composition of unstimulated saliva, with predominant phosphate buffer, were much more important than the same parameters in stimulated saliva in their experiment with root caries lesion *in situ*. These results are in accordance with previous investigations¹² of the effects of saliva on caries development, directed too towards the unstimulated saliva parameters. The most important correlation with all demineralized elements was established with the concentration of total phosphates and proteins in unstimulated saliva. It could be concluded that a high level of salivary phosphates can have a potential to reduce demineralization of active progressive caries lesions¹¹. The results obtained in this doctoral dissertation are in accordance with literature data, and our study is a contribution to the generally accepted belief that the value of phosphate buffer is an important parameter which could influence caries incidence.

Zaključak

U grupi ispitanika sa niskim karijes rizikom u obe vrste pljuvačke uočene su značajno više vrednosti fosfata nego u grupi sa visokim karijes rizikom. Veći kapacitet fosfatnog pufera u stimulisanoj pljuvački obe ispitivane grupe pacijenata, ukazuje na značajnu pufersku aktivnost fosfatnog pufera nestimulisane pljuvačke. Nivo fosfatnog pufera pokazuje negativnu korelaciju sa incidencom karijesa

Conclusion

In the group of examinees with low caries risk in both stimulated and unstimulated saliva groups significantly higher phosphate values were observed compared to the high caries risk group. A higher capacity of phosphate buffer in stimulated saliva in both studied patient groups indicate significant buffer activity of phosphate buffer in unstimulated saliva. The level of phosphate buffer is negatively correlated with caries incidence.

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