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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 ± 3.92 mmol/L and in special tubes 7.90 ± 4.33 mmol/L. Potassium level in unstimulated saliva collected in test tubes was 13.62 ± 0.99 mmol/L while in special tubes it was 13.54 ± 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 ± 1.05 mmol/L) compared to the samples taken by direct spitting into the test tube (1.38 ± 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 ± 0.32, and after direct spitting into test tubes it was 7.35 ± 0.41. Buffer capacity of saliva in healthy subjects was lower after taking with special tubes (5.18 ± 0.74) compared to test tubes (5.36 ± 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

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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 METODS

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 ± 4.33 mmol/L) and potassium (13.54 ± 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 ± 1.05 mmol/L) then by passive spitting into the test tubes (1.38 ± 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 ± SD Srednja vrednost ± SD	p value p vrednost
Sodium (mmol/L) **Natrijum**	Test tube Epruveta	8.43 ± 3.92	p = 0.512
	Special tube Saliveta	7.90 ± 4.33	
Potassium (mmol/L) **Kalijum**	Test tube Epruveta	13.62 ± 0.99	p = 0.414
	Special tube Saliveta	13.54 ± 0.94	
Calcium (mmol/L) **Kalcijum**	Test tube Epruveta	1.38 ± 1.18	p = 0.026*
	Special tube Saliveta	2.04 ± 1.05	
pH value pH vrednost	Test tube Epruveta	7.35 ± 0.41	p = 0.102
	Special tube Saliveta	7.05 ± 0.32	
Buffer capacity (BC) Puferski kapacitet (PK)	Test tube Epruveta	5.36 ± 0.85	p = 0.284
	Special tube Saliveta	5.18 ± 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 ± 0.85, slightly higher than the average value of the buffer capacity 5.18 ± 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

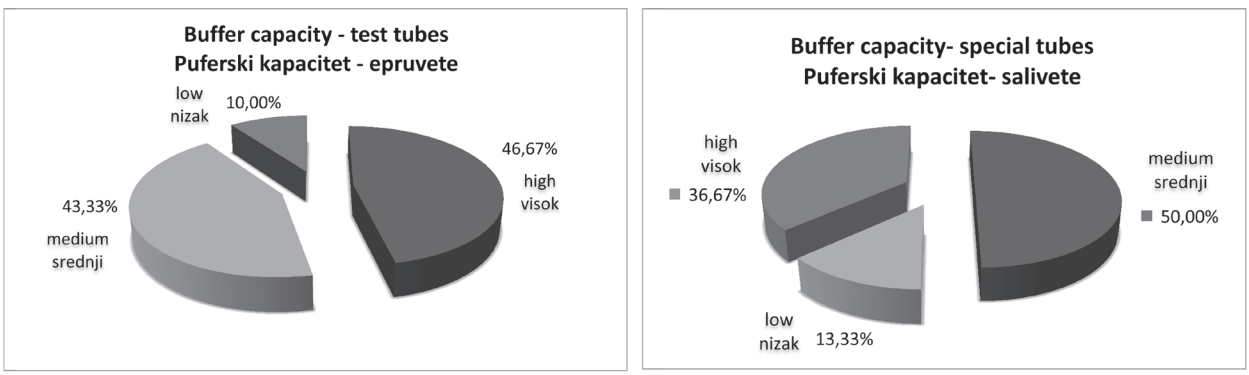

Figure 2. Buffer capacity of saliva samples in healthy subjects
Slika 2. Puferski kapacitet u uzorcima pljuvačke zdravih ispitanika

(citrates) and salivary proteins (staterin, 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 salivar 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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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 ± 3,92 mmol/L, a u salivetama 7,90 ± 4,33 mmol/L. Koncentracija kalijuma u nestimulisanoj pljuvački sakupljenoj epruvetama iznosila je 13,62 ± 0,99 mmol/L, a u salivetama 13,54 ± 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 ± 1,05 mmol/L) u odnosu na uzorak koji je uzet direktnim ispljuvavanjem u epruvetu (1,38 ± 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 ± 0,32, a direktnim ispljuvavanjem u epruvete 7,35 ± 0,41. Puferski kapacitet pljuvačke zdravih ispitanika je bio niži nakon uzimanja salivetama (5,18 ± 0,74) nego epruvetama (5,36 ± 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; elektoliti; 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, hlora, bikarbonata, a smanjuje koncentracija kalijuma i fosfata u odnosu na nestimulisanu pljuvačku [6].

Za održavanje acidobazne ravnoteže u pljuvački najznačajniji su bikarbonantni, 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 medikamenata i psihoaktivnih supstanci [9, 10]. Češće se ispituje nestimulisana pljuvačka, u odnosu na stimulisanu, 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 gumu 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 slivanjem 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 sakupljani 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 ± 4,33 mmol/L) i kalijuma (13,54 ± 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 ± 1,05 mmol/L) u odnosu na pasivno ispljuvavanje u epruvete (1,38 ± 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 ± 0,85 i bila je nešto veća u odnosu na srednju vrednost puferskog kapaciteta 5,18 ± 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 uzoraka 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 prihvaćene 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, mioglobina [26], alfa-amilaze [27], laktoferina [28].

U ovom istraživanju postavljen je cilj da se utvrdi validnost 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 ekstraćelijskoj tečnosti. Studije su pokazale da kod oboljenja pljuvačnih žlezda (Sjögren sindrom), zbog poremećaja apsorpcije na nivou epitelnih ćelija izvodnih kanalića, koncentracija natrijuma u pljuvački je povišena u odnosu na zdrave ispitanike [31]. Za razliku od natrijuma, kalijum je glavni katjon u intraćelijskoj 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 jonima kalcijuma, koji se nalaze u ravnotežnom odnosu sa istim jonima iz hidroksiapatita zubne gleđi. U pljuvački se kalcijum nalazi uglavnom u jonskom obliku (oko 50%), a ostatak u kompleksu sa organskim jonima (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 mmol/L) u odnosu na sakupljanje uzoraka salivetama (2,04 mmol/L). Ovo bi se moglo objasniti činjenicom da je tokom uzimanja pljuvačke pamučnim uloščima 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 biohemijske 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 sniženje 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 pomoću 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).