The Effect of Long-Term Antihypertensive Therapy on the Change in Secretion and Calcium, Bicarbonate and Phosphate Ion Concentration in Non-Stimulated and Stimulated Saliva

Učinak dugotrajne antihipertenzivne terapije na promjenu izlučivanja i koncentraciju kalcijevih, bikarbonatnih i fosfatnih iona u nestimuliranoj i stimuliranoj slini

**Introduction**

Saliva has several different types of functions (antibacterial properties, digestion, cleansing, buffering, lubrication, etc.) which play an important role in maintaining both oral and overall health of the body (1,2). Saliva secretion is controlled by three basic centers; primary (localized in the medulla oblongata), secondary (thalamus), and tertiary salivation center (opercula-insular zone of the cerebral cortex) (3). Based on the manner of production and excretion, mixed saliva is divided into stimulated saliva and resting saliva. Stimulated mixed saliva occurs as a result of the effect of the most diverse fac-

**Uvod**

Slika ima više različitih funkcija (antibakterijska, probavna, čišćenje, puferiranje, podmazivanje, itd.) koje su važne u održavanju zdravlja usne šupljine i cjelokupnog организма (1, 2). Izlučivanje sline kontroliraju tri osnovna centra: primarni (smješten u produženoj moždini), sekundarni (u talamosu) i tercijarni centar za salivaciju (operculo-insularna zona moždane kore) (3). Na temelju načina stvaranja i izlučivanja, sveukupno stvorena slika dijeli se na stimuliranu i nestimuliranu. Stimulirana slika nastaje kao rezultat utjecaja različitih čimbenika koji uzrokuju njezino pojačano izluči-
tors, which act directly in the oral environment on numerous and diverse receptors, and/or indirectly through the sense of sight, hearing or smell, thus causing its secretion to be enhanced. Resting mixed saliva is produced as a secretion product of the entire glandular apparatus of the oral cavity in conditions when there is no external stimulus for its creation.

The acidity of the oral cavity environment is closely related to the amount and the chemical composition of the secreted mixed saliva. The pH measurement of mixed saliva has shown a wide range from the most acidic (pH=6.1) to alkaline (pH=7.8). Acidity of saliva is also associated with the measurement period (during day or night), as well as with the volume of salivary secretion, i.e. whether it is stimulated or resting salivation and it is important in patients undergoing radiotherapy (4,5).

Bicarbonate buffer is excreted in enhanced concentration during stimulated salivation when it represents the dominant buffer. Due to the multiplier effect of enhanced concentration of this buffer, mixed saliva becomes alkaline, reaching a pH of 7.8. In such alkalization conditions, and due to the effect on the acid products present in saliva, it can be considered as a precautionary measure in preventing demineralization of the hard-dental tissues’ surface (1, 5).

Phosphate buffer is the basis of the buffer system in resting saliva. It is extremely important in the physiological processes of resting saliva (during 22 hours in a day), and in the period when there is no stimulated salivary secretion. For remineralization processes of the surfaces of hard-dental tissues, apart from the buffer, the amount of calcium and phosphate ions present in the saliva is pivotal (6,7). A high concentration of these ions in saliva prevents the demineralizing effect of water while remineralizing the enamel’s damaged surface at the same time.

Different local and systemic diseases may be the reason for qualitative and quantitative changes in salivary secretion (Parkinson’s disease, Alzheimer’s disease, glandular infections, tumors and use of certain drugs) (8, 9). The drugs that most commonly cause problems in the qualitative and quantitative secretion of saliva belong to different groups such as antihypertensives, antihistamines, opioids, and antidepressants (10, 11).

Antihypertensives may be divided into two broad groups: the first group of drugs which directly or indirectly block the renin-angiotensin system (the predominant effect is to cause vasodilatation) and the second group of drugs works by increasing water and sodium excretion (diuretics and calcium channel blockers). Different groups of drugs (i.e. inhibitors of the angiotensin converting enzyme, angiotensin receptor blockers, calcium channel blockers, thiazide diuretics), in addition to the antihypertensive effect, can also have an effect on other systemic parts of the human body, as well as on the qualitative and quantitative properties of the stimulated and non-stimulated saliva (12).

This study aimed to determine changes in the amount of saliva excreted and calcium, bicarbonate and phosphate ion concentration in stimulated and resting saliva in patients on long-term antihypertensive therapy (longer than five years), and to compare them with findings in a healthy population.
Material and methods

The study included 62 subjects divided into two groups: patient and control. The patient or experimental group included 31 (18 males, 13 females) subjects who were admitted to a cardiovascular clinic and who had been receiving antihypertensive drug therapy for more than five years. Twelve patients used a combination of beta-adrenergic blockers with ACE inhibitors, ten patients were on beta-adrenergic blockers and diuretics, while the remaining nine patients used a calcium antagonist in therapy of hypertension. The control group included 31 healthy subjects (13 males, 18 females). The study was conducted at the Clinic center and the Biological laboratory of the Faculty of Natural Sciences and Mathematics University of Banja Luka.

The patient group inclusion criteria were as follows: patients diagnosed with hypertension, patients who had been only on antihypertensive therapy for five years or more, hospitalized patients under medical supervision, patients without disease or clinical condition that could affect the qualitative and quantitative characteristics of salivation.

The patient group exclusion criteria were as follows: patients with occasional manifestations of hypertension, patients receiving the antihypertensive therapy occasionally or those receiving it less than five years, patients with disease or clinical condition that could affect the qualitative and quantitative characteristics of salivation.

The control group inclusion criteria were as follows: healthy patients with physiological pressure; patients who were not receiving any therapies.

The control group exclusion criteria were as follows: patients taking occasional antihypertensive drugs or any therapy that may affect the parameters being evaluated.

Each of the subjects received an information form, based on which they voluntarily consented for participation. The study was approved by the Ethics Committee of the Clinic center in Banja Luka, under the no. 01-5-355.2/12, and adhered to the principles outlined in the Declaration of Helsinki.

Sample collection

After the health history had been taken and processed, the external and internal examinations were completed, and the resting and stimulated saliva sampling was done.

Non-stimulated and stimulated saliva samples of the patient were collected before breakfast, without taking any food or drinks previously, and without oral hygiene between 6:30 a.m. and 7:30 a.m. While preparing to take a sample, the patient was seated in a chair with the head slightly tilted forward, with relaxed arms and shoulders.

Non-stimulated saliva was collected in the mouth for five minutes, spat into a sterile measuring cylinder and closed. Each sample was marked with the letter N (non-stimulated, i.e. resting) and identified by an ordinal number from 1 to 31.

Stimulated saliva was collected from patients after they had been chewing a paraffin ball (8 mm in diameter) for 5 minutes, thus stimulating saliva secretion. The saliva obtained by stimulation was spat into a sterile measuring cyl-

Materijal i metode

Studija je obuhvatala 62 ispitanika koji su bili razvrstani u dvije skupine – u prvoj su bili pacijenti, a druga je bila kontrolna. Pacijent ili eksperimentalna skupina obuhvatale je 31 ispitanika (18 muškaraca, 13 žena) koji su bili hospitalizirani u Kardiovaskularnoj klinici i uzimali su antihipertenzivnu terapiju lijekovima pet i više godina. Dvanastioštorci bolesni-
ka propisana je kombinaciju beta-adrenergičkih blokatora s ACE inhibitorima, dijetet je uzimalo beta-adrenergičke blok-
tore i diuretike, a preostalih devet antagonist kalcija u terapiji hipertenzije. Kontrolna skupina obuhvatale je 31 zdravu oso-
bu (13 muškaraca, 18 žena). Istraživanje je provedeno u Kli-
ničkom centru i u Biološkom laboratoriju Prirodno-matemati-
tičkog fakulteta Sveučilišta u Banjoj Luci.

Kriteriji za uključivanje u prvu skupinu bili su: pacijen-
 ti s dijagnozom hipertenzije, pacijenti koji su pet i više godi-\n na bili na antihipertenzivnoj terapiji, hospitalizirani pacijenti pod lijecnickim nadzorom, pacijenti bez bolesti ili kliničkoga stanja koji bi mogli utjecati na kvalitativne i kvantitativne karakteristike izlučivanja sline.

Kriteriji za izuzeće iz skupine bolesnika bili su sljedeći: pacijenti s povremenim nalazima hipertenzije, pacijenti kojima je ordinirana antihipertenzivna terapija povremeno ili kraće od pet godina, bolesnici s bolešću ili kliničkim stanjem koje može utjecati na kvalitativne i kvantitativne značajke izlučivanja sline.

Kriteriji za uključivanje u kontrolnu skupinu bili su: zdravi ispitanici s fiziološkim tlakom, ispitanici koji nisu ni na kakvoj terapiji.

Kriteriji za izuzeće iz kontrolne skupine bili su sljedeći: pacijenti koji povremeno uzimaju antihipertenzivne lijekove, ili bilo koju terapiju koja može utjecati na parametre istraži-
vanja u studiji.

Svaki ispitanik dobio je informativni obrazac te je potpi-
som potvrdo dobrovoljno sudjelovanje u studiji. Studiju je, poštujući načela Helsinške deklaracije, odobrilo Etičko po-

vjerstvo Kliničkoga centra u Banjoj Luci pod brojem 01-

5-355.2/12.

Prikupljanje uzoraka

Nakon provjere anamnestičkih podataka te završetka ek-
straoralnog i intraoralnog pregleda, uzeti su uzorci stimu-
lirane sline.

Uzorci nestimulirane sline uzimani su od is-
pitanika prije doručka, između 6,30 i 7,30 sati, prije čega ni-
su smjeli ništa ni pojesti ni popiti, niti obaviti higijenu usne
špuljine. Tijekom pripreme za uzimanje uzorka pacijent je
sjedio na stolcu s glavom lagano nagnutom prema naprijed te
s opuštenim rukama i ramenima.

Nestimulirana slika skupljala se u ustima pet minuta te
zatim ispljunula u sterilni mjerni cilindar i zatvorila. Svak
i je uzork označen slovom N (nestimulirana) i rednim bro-
jem od 1 do 31.

Stimulirana slika skupljena je od ispitanika nakon što su
5 minuta žvakali parafinsku kuglicu (promjera 8 mm), sti-
mulirajući tako izlučivanje sline. Slika dobivena stimulacijom

ispljunuta je u sterilni mjerni cilindar i zatvorena. Uzorci do-

biveni od svakog ispitanika označeni su brojevima od 1 do 31.
inder and closed. The samples obtained were marked with numbers from 1 to 31 and with letter S (stimulated) for each patient.

During their transport to the laboratory (10 minutes), the samples were stored in a mobile refrigerator at a temperature of 4 °C until analyzed. The saliva sample obtained was poured into a glass measuring cylinder (graduated by 1 ml).

Sample analysis

The measured amount of saliva was recorded and divided by 5 to obtain the value of a milliliter of saliva per minute, and the final result was recorded in the questionnaire prepared. The measured amount of saliva was further used to determine the calcium, phosphate and bicarbonate ion concentration values. Calcium and phosphate ions were determined spectrophotometrically, while bicarbonate ions were determined by titration.

Calcium ion concentration was determined by the reaction of saliva sample and calcium arsenazo III reagent (2,2′-[(1,8-Dydroxy-3,6-disulfonylphenylene-2,7-bisazo)] bisbenzenearsonic acid), 2,7-Bis (2-aronophenylazo) chromotropic acid, in such a way that 1 ml arsenazo III reagent was added to 10 µL of saliva, mixed and left for 3 minutes at room temperature (20-25 °C). After that, spectrophotometric measurement was performed with UV -1800 Shimadzu Spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 650 nm wavelength and obtained values were read on a spectrophotometer. The color intensity was directly proportional to calcium concentration in the saliva sample. The obtained values were entered in a formula (13):

\[
\text{calcium concentration} = \frac{\text{SA Abs}}{\text{ST Abs}} \times 10 = \text{mg calcium/dL}
\]

- sample absorbance x 10
- standard absorbance
- SA Abs = sample absorbance
- ST Abs = standard absorbance
- The obtained values were tabulated.

The phosphate concentration was determined by molybdenum reaction method of by Goldberg and Fernandez's spectrophotometric method, modified by Bardow et al. (13). Buffer (reaction mixture consisting of 10% trichloroacetic acid (TCA), 1% urea and 3% Mohr's salt) was added to saliva. The buffer was prepared directly in the laboratory. After 10 minutes, centrifugation was performed (Tehtnica Centric 200R, Domel, Železniki, Slovenia) at 5000 rpm. It separates the supernatant and adds concentrated sulfuric acid and 4.5% ammonium molybdate to the supernatant. The preparation was kept for 20 minutes at room temperature and then the absorbance was spectrophotometrically measured at 700 nm. The standard curve was made in the range from 0 to 10 µmol / L of phosphate.

Bicarbonate concentration is determined by titration with 0.1M HCl ranging from pH 7 to pH 3, by adding HCl to the sample in a volume of 100 µL. Titrated saliva was measured in the said solution and each value was recorded. After the measurement, it was entered into the formula and final value was obtained. The formula used for calculating bicarbonate was:

\[
K1 = \frac{C1V1}{C2V2}
\]

Analiza uzoraka

Izmjerena količina sline zabilježena je i podijeljena s 5 da bi se dobila vrijednost miliitra izlučene sline u minuti, a konačni rezultat zabilježen je u pripremljenom upitniku. Izmjrena količina sline dalje je korištena za određivanje vrijednosti koncentracije kalcijevih te fosfatnih i bikarbonatnih iona. Koncentracija kalcijevih i fosfatnih iona određivala se spektrofotometrijski, a koncentracija bikarbonatnih iona titracijom.

Koncentracija kalcijevih iona određena je reakcijom uzorka sline i kalcijeva arsenazo III reagensa [2,2′ - (1,8-dihidrokiso-3,6-disulfonyliden-2,7-bisazo)] bisbenzenarsionska kiselina te 2,7-Bis (2-aronofenilazo) kromotropne kiseline, na način da se 1 mL arsenazo III reagensa doda u 10 µL sline, pomiješa i ostavi tri minute na sobnoj temperaturi (20 – 25 °C). Nakon toga provedeno je mjerenje spektrofotometrom UV-1800 Shimadzu (Shimadzu Corporation, Kjoto, Japan) na valnoj duljinii od 650 nm na kojem su očitan dobivena vrijednosti. Intenzitet boje izravno je proporcionalan koncentraciji kalcija u uzorku sline. Dobivene vrijednosti unese su u formulu (13):

\[
\text{Koncentracija kalcija} = \frac{\text{SA Abs}}{\text{ST Abs}} \times 10 = \text{mg kalcija/dL}
\]

- SA Abs = apsorbancija uzorka
- ST Abs = standardna apsorbancija
- Dobivene su vrijednosti prikazane u tablicama.

Koncentracija fosfata odredila se molibdskom reakcijom prema spektrofotometrijskoj metodi Goldenberga i Fernandeza modificiranoj prema Bardowu i suradnicima (13). Slini se dodao pufer [reakcijska smjesa koju čini 10-postotna trikloroasencna kiselina (TCA), 1-postotna urea i 3-postotna Mohrova sol]. Pufer je pripremljen u laboratoriji neposredno prije primjene. Nakon 10 minuta obavljeno je centrifugiranje (Tehtnica Centric 200R, Domel, Železniki, Slovenija) na 5000 okretaja, odvojen je supernatant kojemu je dodana koncentrirana sumporna kiselina i 4,5-postotni amonijev molibdat. Pripremak je držan 20 minuta na sobnoj temperaturi te su zatim spektrofotometrijski određene vrijednosti apsorpcije u području od 700 nm. Standardna krivulja izrađena je u rasponu od 0 do 10 µmol/L fosfata.

Koncentracija bikarbonata određena je titracijom s 0,1 M HCl-a u rasponu od pH 7 do pH 3, dodavanjem korovodčine kiseline uzorku u volumenu od 100 µL. U spomenutoj otopini izmjerena je titrirana slina i zabilježena je dobivena vrijednost. Nakon mjerenja su dobivene vrijednosti unese u formulu i dobivena je konačna vrijednost. Formula korištena za izračunavanje bikarbonata bila je:

\[
K1 = \frac{C1V1}{C2V2}
\]
C¹V¹=C²V²

C¹ – acid concentration
C² – the unknown value that we are looking for,
V¹ – total titration agent volume,
V² – total volume (saliva and titration agent)

We divided the obtained C² value by ΔpH, which represents the difference between the first pH sample and the last pH sample measured. Values were expressed in mmol/L. All values obtained from saliva samples were documented in the questionnaire for each patient individually.

Statistical analysis

A two-way-test (Student’s test) was used to compare the values of the variables under the following assumptions: the variances of the compared data groups are unequal and the difference of the mean values of the variables being compared is equal to zero. The differences were considered significant at P<0.05. Standard descriptive statistics tools (frequency tables, grouping of data into classes, graphs, calculation of arithmetic mean and minimum and maximum values, standard deviation and variance) were used to display and analyze the data obtained by the research.

Results

The values obtained by measuring the amount of saliva and calcium, phosphate and bicarbonate ion concentration in patient and control subjects in stimulated and resting saliva are presented in Tables 1-4 and Figures 1-4.

The average amount of saliva of the stimulated and resting saliva in patient and control subjects is presented in Table 1 and Figure 1. The results obtained show a statistically significant difference in the amount of non-stimulated saliva excreted between the subjects from the patient (1.739 mL/5 min) and control groups (3.535 mL/5 min) (p: 0.00018, p<0.05), as well as in stimulated saliva where there is a statistically significant difference between the two groups (3.594 mL/5 min / 6.271 mL/5 min) (p: 0.00048, p<0.05).

The calcium concentration values in stimulated and non-stimulated saliva in patient and control groups are presented in Table 2 and Figure 2. In non-stimulated saliva, there is a statistically significant difference between the average values of patient (6.143 mg/dL) and control subjects (7.922 mg/dL) (p: 0.03, p<0.05), while there is no statistically significant difference between the control groups.

Rezultati

Vrijednosti dobivene mjerenjem količine koncentracije sline te kalcijevih, fosfatnih i bikarbonatnih iona bolesnika i ispitanika u kontrolnoj skupini, u stimuliranoj i nestimuliranoj slini prikazane su u tablicama 1 do 4 i na slikama od 1 do 4.

Prosječna količina stimulirane i nestimulirane sline pacijenata i sudionika u kontrolnoj skupini prikazana je u tablici 1. i na slici 1. Dobiveni rezultati pokazuju statistički značajnu razliku između prosječnih vrijednosti dobivenih od pacijenata (1,739 mL/5 min.) i kontrolne skupine (3,535 mL/5 min.) (p: 0,00018, p < 0,05) te u količini stimulirane sline gdje postoji statistički značajna razlika između tih dviju skupina (3,559 mL/5 min.; 6,271 mL/5 min.) (p: 0,00048, p < 0,05).

Vrijednosti koncentracije kalcijevih, fosfatnih i bikarbonatnih iona bolesnika i ispitanika u kontrolnoj skupini prikazane su u tablicama od 1 do 4 i na slikama od 1 do 4.

Statistička analiza

Za usporedbu vrijednosti varijabli korišten je dvosmjerni test (Studentov test), a pretpostavke su bile sljedeće: varijance uspoređenih skupina podataka nejednake su i razlika srednjih vrijednosti varijabli koje se uspoređuju jednaka je nuli. Razlike su se smatrali značajnima kada je P < 0,05. Za prikaz i analizu podataka dobivenih istraživanjem korišteni su standardni opisni statistički alati (tablice učestalosti, grupiranje podataka u razrede, grafovi, izračun aritmetičke sredine te najniže i najviše vrijednosti, standardno odstupanje i varijanca).

| Table 1 | Amount of excreted saliva. |
|---------|----------------------------|
| Non-stimulated saliva | | Stimulated saliva |
| | Experimental | Control | Experimental | Control |
| Saliva amount (ml/5 min) | (N) | (N) | (S) | (S) |
| Total number | 31 | 31 | 31 | 31 |
| Average value | 1.739 | 3.535 | 3.594 | 6.271 |
| Min value | 0.400 | 1.000 | 0.500 | 2.200 |
| Max value | 5.800 | 8.300 | 11.000 | 11.200 |
| Sr. deviation | 1.30810 | 1.82365 | 2.73982 | 2.63947 |
| Variation coefficient | 75.23% | 51.58% | 76.24% | 42.097% |
| t value | 0.000108 | 0.00048 |
Table 2  Calcium ion concentration value in stimulated and non-stimulated saliva excreted.

| Non-stimulated saliva | Stimulated saliva |
|-----------------------|------------------|
| **Ca (mg/dl)** | **Experimental (N)** | **Control (N)** | **Experimental (S)** | **Control (S)** |
| Total number | 31 | 31 | 31 | 31 |
| Average value | 6.143 | 7.922 | 5.911 | 6.541 |
| Min value | 0.917 | 0.464 | 1.409 | 3.217 |
| Max value | 14.173 | 13.805 | 12.987 | 12.481 |
| St. deviation | 3.53591 | 2.87808 | 3.10942 | 2.11706 |
| Variation coefficient | 57.56% | 36.33% | 52.60% | 32.37% |
| **t** value | 0.033934 | 0.355793 |

Table 3  Phosphate ion concentration value in stimulated and non-stimulated saliva.

| Non-stimulated saliva | Stimulated saliva |
|-----------------------|------------------|
| **Phosphates (µmol/l)** | **Experimental (N)** | **Control (N)** | **Experimental (S)** | **Control (S)** |
| Total number | 31 | 31 | 31 | 31 |
| Average value | 2.818 | 1.388 | 1.454 | 0.565 |
| Min value | 0.109 | 0.054 | 0.018 | 0.018 |
| Max value | 17.636 | 3.254 | 5.927 | 3.690 |
| St. deviation | 3.51657 | 1.07435 | 1.47937 | 0.77418 |
| Variation coefficient | 124.79% | 77.41% | 101.77% | 137.02% |
| **t** value | 0.037135 | 0.004839 |

Table 4  Bicarbonate ion concentration value in stimulated and non-stimulated saliva.

| Non-stimulated saliva | Stimulated saliva |
|-----------------------|------------------|
| **Bicarbonates (mmol/l)** | **Experimental (N)** | **Control (N)** | **Experimental (S)** | **Control (S)** |
| Total number | 31 | 31 | 31 | 31 |
| Average value | 14.041 | 9.929 | 10.872 | 5.964 |
| Min value | 2.939 | 2.890 | 2.562 | 2.467 |
| Max value | 37.131 | 29.600 | 38.300 | 10.219 |
| St. Deviation | 9.48100 | 9.07875 | 9.41743 | 2.47299 |
| Variation coefficient | 67.53% | 51.15% | 86.59% | 41.47% |
| **t** value | 0.03870 | 0.00819 |

cant difference between the two groups in stimulated saliva 
(5.911 mg/dL / 6.541 mg/dL) (p: 0.35, p>0.05).

The average phosphate concentration values in non- 
stimulated and stimulated saliva in patient and control subjects are presented in Table 3 and Figure 3. In non-stimulated 
(2.818 mmol/L / 1.388 mmol/L) (p: 0.03, p<0.05) and stimulated saliva (1.454 mmol/L / 0.565 mmol/L) (p: 0.004, p<0.05) there are statistically significant differences in the av-

Table 3. Vrijednost koncentracije fosfatnih iona u stimuliranoj i nestimuliranoj slini

Table 4. Vrijednost koncentracije bikarbonatnih iona u stimuliranoj i nestimuliranoj slini

The bicarbonate concentration value in resting and stim-
ulated saliva in patient and control groups is presented in 
Table 4 and Figure 4. In non-stimulated (14.041 mmol/L

ka u kontrolnoj skupini (7,922 mg/dL / 6,541 mg/dL) (p: 0.03, p < 0.05), a
nema statistički značajne razlike između dviju skupina u sti-
muliranoj slini (5,911 mg/dL ; 6,541 mg/dL) (p: 0,35, pa>0,05).

Prosječne vrijednosti koncentracije fosfata u nestimuliranoj

ka i stimuliranoj slini pacijenata i sudionika u kontrolnoj

ka skupini prikazane su u tablici 3 i na slici 3. U nestimuliranoj

ka slini (2,818 mmol/L; 1,388 mmol/L) (p: 0,03, p < 0,05) i

stimuliranoj (1,454 mmol/L ; 0,565 mmol/L) (p: 0,004, p < 0,05) zabilježene su statistički značajne razlike u prosječnim vrijednostima između pacijenata i sudionika u kontrolnoj

ka skupini.
In non-stimulated saliva (14.041 mmol/L; 9.929 mmol/L) (p: 0.03, p < 0.05) and in stimulated saliva (10.872 mmol/L / 5.964 mmol/L) (p: 0.008, p<0.05), there are statistically significant differences in the average values between the patient and control group subjects.

Discussion

The qualitative and quantitative features of saliva are related to the biological functions of the entire body and may be related to the emergence and development of both local and several systemic diseases (14-16). The amount of resting saliva in a healthy person under physiological conditions ranges from 0.3 ml/min to 0.4 mL/min. In the case of reduced saliva secretion (oligosalammum), the amount of saliva excreted ranges from 0.2 to 0.4 mL/min, and if the values of

Vrijednost koncentracije bikarbonata u nestimuliranoj i stimuliranoj slini pacijenata i sudionika u kontrolnoj skupini prikazana je u tablici 4 i na slici 4. U nestimuliranoj sli

Rasprava

Kvalitativni i kvantitativni nalazi sline povezani su s biološkim funkcijama cijelog organizma te mogu biti poveza

ni s pojavom i nastankom kako lokalnih tako i pojedinih si
temska bolesti (14 – 16). Količina nestimulirane sline kod zdrave osobe, u fiziološkim uvjetima, kreće se u vrijedno
stima od 0.3 ml/min do 0.4 ml/min. U slučaju smanje
nog izlučivanja sline (oligosalajja), količina izlučene sline je od 0,2 do 0,4 ml/min. a ako su vrijednosti izlučivanja sli-
saliva excretion are less than 0.2 mL/min that is called hyposalivation (17., 18.).

In this study, a statistically significant difference (p=0.000108) was found in the average values of the resting saliva excreted in the patient group (1.739 mL/ 5 min) compared to the control group (3.535 mL/5 min). Moreover, a statistically significant difference (p=0.00048) of the average values of stimulated saliva excreted in the patient group (3.594 mL/5 min), compared to the control group (6.271 mL/5 min). These results are consistent with those of Leandro (19), who measured the amount of saliva excreted in patients using beta blockers. Kagawa et al. (20) obtained slightly different results in which there was no difference in the amounts of resting and stimulated saliva excreted in patient and control group. Consistent with our study, Nauntofte and Twetman (21) reported an increase in xerostomia in patients on diuretic therapy. The reduced amount of resting and stimulated saliva excreted in the patient group may be related to the long-term usage of antihypertensive medications, as confirmed by Murray (22) in his study.

Skanda (23) confirmed that there was a close relation between the use of thiazide medication and xerostomia, stating that xerostomia was ten times more enhanced after furosemide ingestion. Cano et al. (24) suggested that renin-angiotensin system mechanisms compelling exclusively vasoconstriction and dilation are not able to completely describe salivary glands local mechanisms on saliva release, since the decrease of angiotensin converting enzyme expression in myoepithelial cells cannot explain lower salivary rates by itself. They considered that local angiotensin converting enzyme, in addition to other cascades, would affect these cells contraction and further reduce saliva flow. In this study, no difference was observed in the administration of certain medications from different groups of antihypertensives in the saliva obtained.

The amount of saliva excreted as well as its physicochemical properties (composition, pH value), with particular emphasis on calcium and phosphate concentrations, are closely related to the emergence of demineralization and carious lesions in the hard-dental tissue area (25, 26). Calcium concentration values in saliva, on average, under physiological conditions in a healthy person are 8.8-10.5 mg/dL. This study obtained a statistically significant (p=0.034) average calcium concentration value in resting saliva in the patient group (6.143 mg/dL) compared to control group (7.922 mg/dL). Stojšin (27) obtained approximately the same values for average calcium concentration in resting saliva. The average calcium concentration value in stimulated saliva in the patient group was 5.911 mg/dL, while in the control group it was 6.541 mg/dL. Although lower values were obtained in the patient group, they were not statistically significant. By comparing the average calcium concentration values in stimulated and resting saliva, higher values were shown in resting saliva in these two groups. Contrary to the results of this study, Gauri, Nagarajappa, Bhat (28) found a lower average value of 5.87 mg/dL in resting saliva, whereas after stimulation, the average calcium concentration value was 7.17 mg/dL. Jarvinen (29) in his study obtained a lower calcium and ne manje od 0.2 mL/min., takvo se stanje naziva hiposalivacija (17., 18.).

U ovom istraživanju utvrđena je statistički značajna razlika (p = 0,000108) u srednjim vrijednostima količine izlučene nestimulirane sline u skupini pacijenata (1,739 mL/5 min) u odnosu prema kontrolnoj skupini (3,535 mL/5 min.). Ta-kođer je utvrđena statistički značajna razlika (p = 0,00048) u srednjim vrijednostima količine izlučene stimulirane sline u skupini pacijenata (3,594 mL/5 min.) u usporedbi s kontrolnom skupinom (6,271 mL/5 min.). Ti su rezultati u skladu s rezultatima Leandra (19) koji je mjerio količinu izlučene sline u skupini pacijenata koji su se koristili beta-blokatorima. Kagawa i suradnici (20) dobili su nešto drukčije rezultate – nije bilo razlike u količini izlučene nestimulirane i stimulirane sline kod pacijenata i sudionika u kontrolnoj skupini. U skladu s našim istraživanjem, Nauntofte i Twetman (21) izviđestio su o porastu kserostomije kod pacijenata na terapiji diuretikima. Stojšin količina nestimulirane i stimulirane sline zabilježena u skupini pacijenata može biti povezana s dugotrajnom primjenom antihypertenzivnih lijekova, što je u svojoj studiji potvrdio Murray (22).

Skanda (23) je istaknuo usku povezanost između primjene tiazidnih lijekova i kserostomije, navodeći da je kserostomija deset puta pojačana nakon uzimanja furosemida. Cano i suradnici (24) sugeriraju da mehanizmi renin-angiotenzinskog sustava koji počivaju isključivo na vazokontrakciji i dilataciji nisu u stanju potpuno opisati lokalne mehanizme izlučivanja sline u žljede slinovnicima, zato što smanjenje ekspresije enzima pretvarača angiotenzina u mioepitelnim stanicama samo po sebi ne može objasniti smanjene vrijednosti u izlučivanju sline. Oni smatraju da bi lokalni enzimi koji djeluju na pretvorbu angiotenzina, uz ostale kaskade, mogao utjecati na kontrakciju tih stanica i dodatno smanjiti protok sline. U ovom istraživanju nije uočena razlika pri primjeni pojedinih lijekova iz različitih skupina antihipertenziva na količinu izlučene sline.

Količina izlučene sline i njezina fizikalno-kemijska svojstva (sastav, pH vrijednost), s posebnim naglaskom na koncentraciju kalcija i fosfata, usko su povezana s pojavom demineralizacije i karijskih lezija u području tvrđih zubnih tkiva (25, 26) .

Vrijednosti koncentracije kalcija u slini, u fiziološkim uvjetima i kod zdrave osobe, prosječno iznose od 8,8 do 10,5 mg/dL. U ovom istraživanju dobivena je statistički značajna (p = 0,034) prosječna vrijednost koncentracije kalcija u nestimuliranoj slini u skupini pacijenata (6,143 mg/dL) u usporedbi s kontrolnom skupinom (7,922 mg/dL). Stojšin (27) je utvrdila približno iste vrijednosti prosječne koncentracije kalcija u nestimuliranoj slini. Prosječna vrijednost koncentracije kalcija u nestimuliranoj slini u skupini pacijenata bio je 5,911 mg/dL, a u kontrolnoj skupini iznosila je 6,541 mg/dL. Iako su u skupini pacijenata dobiveni niži vrijednosti, one nisu bile statistički značajne. Usporedbom prosječnih vrijednosti koncentracije kalcija u nestimuliranoj i nestimuliranoj slini dobiveni su više vrijednosti u nestimuliranoj slini u objema ispitivanim skupinama. Suprotno rezultatima u ovoj studiji, Gauri, Nagarajappa i Bhat (28) pronašli su nižu prosječnu vrijednost od 5,87 mg/dL u nestimuliranoj slini, a po-
Phosphate concentration values in resting saliva, which was explained by the small amount of resting saliva in all study groups. Some studies have shown that critical pH value (below 5.5) could be modified if calcium and phosphate substituents were added depending on the amount of saliva excreted and the pH value (30,31).

Phosphate buffer is the dominant buffer in resting saliva. It is a combination of primary and secondary phosphate, where the concentration in resting saliva is 7-8 mM/L, and during the salivation stimulation period, this value decreases to 2-3 mM/L (32).

The results of the current study showed a statistically significant difference in phosphate concentration value between the patient and control groups, with values higher in resting saliva compared to stimulated saliva. The average phosphate concentration value in resting saliva in the patient group was higher (2.818 µmol / L), compared to the control group (1.388 µmol / L) (p<0.05). In stimulated saliva, phosphate concentration values were higher in the patient group (1.454 µmol / L) than in the control group (0.565 µmol / L). The higher phosphate concentration obtained in the patient group saliva could have an impact on reducing the frequency of demineralization processes and carious lesions on hard dental tissues. Šurdilović (33) found that phosphate concentration values were significantly high in both stimulated and resting saliva in children with low caries risk. In assessing phosphate concentration results in resting saliva, the potentially possible part that remains bound and neutralized by acidic products in the oral cavity should also be considered (34,35).

The physiological values of salivary bicarbonates are between 1-60 mM, with the highest values obtained from the parotid and submandibular gland (36). In this study, a statistically significant difference was found between the bicarbonate concentration values obtained in stimulated and resting saliva in both patient and control group. The bicarbonate concentration is higher in resting saliva in both study groups. It amounts to 10.872 mmol/L in the patient group, and 9.929 mmol/L in the control group, while the bicarbonate concentration in stimulated saliva in the patient group is 5.964 mmol/L. During long-term saliva stimulation, although the amount of the excreted saliva increases, the bicarbonate concentration value decreases, with respect to the total amount, thereby depleting its buffering power, regardless of any further stimulation present. An explanation for the low bicarbonate concentration in stimulated saliva, obtained in this study, could be related to the following factors: the sampling of stimulated saliva that was performed in the early morning, where patients did not take any water or food; chewing paraphin without taste that was used as a stimulating agent; and the intensity of the chewing cycles that were done. Bardow et al. (37) pointed out that the amount of bicarbonate in the saliva depends on the total amount of saliva excreted. In their study, Bardow, Nyvad and Nauntofte (38) emphasized that bicarbonate concentration in saliva is dependent on the flow rate and on the intensity of the stimulation applied. They also noticed that different values of the obtained concentrations are related to the number of chewing cycles.

sljede stimulacije je prosječna vrijednost koncentracije kalcija bila 7,17 mg/dL. Jarvinen (29) je u svojoj studiji dobio niže vrijednosti koncentracije kalcija i fosfata u nestimuliranoj sli- ni, što je objašnjeno malom količinom nestimulirane sline u svim ispitivanim skupinama. Studije su pokazale da bi se kri- tična pH vrijednost (niža od 5,5) mogla izmijeniti ako bi se dodali supstituenti kalcija i fosfata, a ovisno o količini izluče- ne sline i njezinoj pH vrijednosti (30, 31).

Fosfatni pufer je dominantan u nestimuliranoj sli- ni. To je kombinacija primarnog i sekundarnog fosfata, gdje koncentracija u stimuliranoj sli- ni iznosi od 7 do 8 mM/L, a tijekom stimulacije salivacije ta se vrijednost smanjuje na 2 do 3 mM/L (32).

Rezultati studije pokazali su statistički značajnu razliku u vrijednosti koncentracije fosfata između pacijenta i kontrolne skupine, s vrijednostima višima u nestimuliranoj sli- ni u usporedbi sa stimuliranom. Prosječna vrijednost koncentracije fosfata u nestimuliranoj sli- ni u skupini pacijenata bila je viša (2.818 µmol/L) u usporedbi s kontrolnom skupinom (1.388 µmol/L) (p < 0.05). U stimuliranoj sli- ni vrijednosti koncentracije fosfata bile su više u skupini pacijenata (1.445 µmol/L) u odnosu prema kontrolnoj skupini (0.565 µmol/L). Veća koncentracija fosfata dobivena u sli- ni skupine pacijenata mogla bi utjecati na smanjenje učestalosti procesa deminerali- zacije i karijskih lezija na tvrdom zubnim tkivima. Šurdilo- vić (33) je utvrdio da su vrijednosti koncentracije fosfata kod djece s niskim rizikom od karijesa bile značajno povišene i u stimuliranoj i u nestimuliranoj sli- ni. Pri procjeni rezultata koncentracije fosfata u nestimuliranoj sli- ni također treba uzeti u obzir mogući dio koji ostaje vezan i neutraliran kiselim spojevima u usnoj šupljini (34, 35).

Fiziološke vrijednosti bikarbonata u sli- ni kreću se izme- du 1 i 60 mM, a najviše vrijednosti dobivene su iz parotidne i submandibularne žližje (36). U ovom istraživanju zabilježi- na je statistički značajna razlika između vrijednosti koncentracije bikarbonata dobivenih u stimuliranoj i nestimuliranoj sli- ni i u skupini pacijenata i u kontrolnoj skupini. Koncentracija bikarbonata veća je u nestimuliranoj sli- ni u objema ispitivanih skupinama i iznosi 10,841 mmol/L u skupini pacijenata i 9,929 mmol/L u kontrolnoj skupini, a koncentracija bikarbonata u stimuliranoj sli- ni u skupini pacijenata je 10,872 mmol/L i u kontrolnoj skupini 5,964 mmol/L. Tijekom du- gotrajne stimulacije, iako se količina izlučene sline poveća, vrijednost koncentracije bikarbonata smanjuje se u odnosu prema ukupnoj količini, čime se iscrpljava njezina puferska moć, bez obzira na daljinu stimulacije. Objasnjenje za nisku koncentraciju bikarbonata u stimuliranoj sli- ni, dobiveno u ovoj studiji, moglo bi se povezati s nekoliko čimbenika – uzimajući u obzir stimulirane sline koje je obavljen, rano uju- tro pri čemu pacijenti nisu uzimali vođu ili hrano, žvakanjem parafin bez okusa koji se koristio kao stimulativno sredstvo i intenzitetom izvedenih ciklusa žvakanja. Bardow i suradnici (37) istaknuli su da količina bikarbonata u sli- ni ovisi o ukupnoj količini izlučene sline. Bardow, Nyvad i Nauntofte (38) u svojoj studiji istaknuli da koncentracija bikarbonata u sli- ni ovisi o brzini protoka i o intenzitetu primijenjenih stimula- cija. Također su uočili različite vrijednosti dobivenih koncen- tracija u odnosu prema broju ciklusa žvakanja.
Qualitative changes, especially a decrease in calcium ion concentration, can greatly affect possible emergence of demineralization and carious lesions on hard dental tissues. On the contrary, the increased phosphate and bicarbonate ion concentration can stimulate the process of remineralization of hard dental tissues.

Conclusions

With the limitation of this study, it can be concluded that in patients who have been on continuous antihypertensive therapy for five years or more, compared to the healthy population, a reduced salivation with calcium ions deficiency can be expected, which can lead to increased demineralization of hard dental tissues, and contribute to plaque formation and carious lesions. In order to prevent such processes, it is necessary to compensate for the lack of excreted amount of saliva and calcium ions by implementation of effective prevention programs. On the contrary, the increase in the phosphate and bicarbonate ion concentration in the patient group affects the regulation of acid-base balance and remineralization process, i.e. hard dental tissue preservation, and thus has a preventiv e effect.

Conflict of interest

There are no conflicts of interest to declare.

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R. A. – koordinirala cijelo istraživanje, sudjelovala u pri kupljanju kliničkih uzoraka, radila statistiku i napisala članak; B. P. – sudjelovala u planiranju studije, pregledao i pripremio članak za objavljivanje; N. T. – sudjelovala u kliničkom dije lu istraživanja i pregledavala odabranu znanstvenu literaturu; O. J. – sudjelovala u kliničkim dijelima istraživanja i analizirala podatke dobivene mjerenjem; V. V. – sudjelovala u pripremi pojedinačnih uzoraka za laboratorijsko istraživanje i analizirala dobivene rezultate.

Kualitativne promjene, posebno smanjena koncentracija kalcijskih iona, uvelike utječu na moguću pojavu demineralizacije i karijesnih lezija na tvrdim zubnim tkivima. Suprot no tomu, povećana koncentracija fosfata i bikarbonatnih ions na može potaknuti proces remineralizacije tvrdoga zubnog tkiva.

Zaključak

Uz ograničenja u ovoj studiji, može se zaključiti da se kod pacijenata koji su pet i više godina na kontinuiranoj antihipertenzivnoj terapiji, a u usporedbi sa zdravom populacijom, može očekivati smanjeno izlučivanje sline i nedostatak kalcijskih iona, što može pojačati nastanak demineralizacije tvrdih zubnih tkiva te pridonijeti većoj mogućnosti stvaranja plaka i karijesnih lezija. U svrhu sprječavanja takvih procesa potrebno je preventivnim programom nadoknaditi nedostatak izlučene količine sline i kalcijskih iona. Suprotno navedenomu, povećanje koncentracije fosfatskih i bikarbonatnih iona u skupini pacijenata utječe na regulaciju acido-bazne ravnoteže i proces remineralizacije, tj. na očuvanje tvrdoga zubnog tkiva te na taj način djeluje preventivno.
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