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LABORATORY TESTING OF UNSTIMULATED AND STIMULATED SALIVA BUFFERING CAPACITY IN PATIENT AND CONTROL GROUP AFTER TITRATION WITH HCl AND NaOH

Radmila Arbutina [1*], Nataša Trtić[1], Ognjenka Janković[1], Valentina Veselinović[1], Irena Radman Kuzmanović[1], Vladan Mirjanić[1], Sanja Gnjato[1], Adriana Arbutina[1]

University of Banja Luka, Faculty of Medicine,
Study Program of Dental Medicine, Banja Luka, RS, B&H
*Corresponding author: radmila.arbutina@med.unibl.org

Abstract: Introduction: Buffer capacity is the "resistance" of saliva to change pH when treated with acid or base. In other words, saliva has a greater buffering capacity to which more acid or base needs to be added in order for its pH to decrease or increase by a certain value.
Aim: Laboratory examination of the buffering capacity of unstimulated and stimulated saliva in patients of the experimental group and subjects of the control group in relation to age and gender.
Material and method: Determination of the pH value of saliva is done with a pH meter (HANNA instruments 8521). The obtained amount of saliva is diluted with distilled water. Each sample is divided into two equal parts, and the pH value of the sample is measured. Saliva titration is done with acid (HCl) and base (NaOH), adding 100µl of HCl to each sample and 100µl of NaOH to each sample, in the range from pH 3 to pH 11.
Results: There is no statistical significance of the differences in the mean values of the buffer capacity.
Conclusion: There is a difference in the mean values of the buffer capacity in favor of men, which explains that in men a larger amount of spent buffer is needed to change the pH by one unit.

Key words: stimulated saliva, unstimulated saliva, buffer capacity, pH value

1. INTRODUCTION

Lack of saliva in the mouth is a serious problem because saliva is a protective factor in the mouth and it helps in self-cleaning of the oral cavity; it neutralizes the acids that create bacteria in the mouth and helps to wash away food residues [1].

Secreted saliva volume and quality depend on the state of oral environment, that is, on the state of the primary salivation center, as well as certain parts of the cerebral cortex. Therefore, saliva or more specifically, mixed saliva can be roughly divided into the so-called unstimulated and stimulated saliva. Unstimulated mixed saliva is produced as a product of secretion of the entire glandular apparatus of the oral environment under non-stimulation conditions, i.e., when no nutritional substances affect the gustatory and other receptors in the oral cavity. Salivary secretion produced under such conditions of non-stimulation is called unstimulated mixed saliva. Stimulated mixed saliva is formed as a logical consequence of the most divers factors' activity, which acting directly in the oral environment on numerous and diverse receptors and indirectly through the senses of sight, hearing and smell, causes increased mixed saliva secretion in a significantly larger than it would be the case without their activity [2].

In the oral environment, saliva maintains oral homeostasis, which means that it maintains biochemical conditions that prevent non-physiological changes in the biochemical composition of all tissues in the oral environment. Simply put, it represents healthy teeth and healthy oral mucosa maintenance [3]. Either as unstimulated or stimulated mixed saliva, with its presence and its ingredients, it enables numerous functions within oral homeostasis, namely: maintaining the humidity of the oral environment, self-cleaning the oral environment, buffering, maintenance and integrity preservation of oral structures, and bacterial flora stabilization in the oral cavity environment.

The current acidity (pH) in the oral environment directly depends on the presence and the amount of mixed saliva. Its pH value measurement showed that there is quite a wide range of these values, from the most acidic pH=6.1 to the alkaline pH=7.8. The pH values certainly depend on measurement time period (day or night), place as well as salivary secretion volume, i.e., whether it is stimulated or unstimulated salivation.

Mixed saliva contains more buffer, it is basically a buffering secretion mechanism which consists of: bicarbonate buffer, phosphate buffer, urea, preventive salivary amylase type of buffer and preventive prophylactic fluoride type of buffer. Phosphates and bicarbonates are ions in the electrolyte saliva composition and are important for maintaining the pH of saliva because they are part of the matching phosphate and bicarbonate buffers. The optimal conditions are realized within the physiological range from 6.1 to 7.8, with saliva's buffering effect joined with the effect of these two buffers, phosphate and bicarbonate, thus preventing the hydroxyapatite dissolution with acidic products' activity in the saliva, which would occur in case the pH decreased to 6.1 [4].

The basic, primary buffering saliva system consists of bicarbonate and phosphate buffer, while buffering saliva system in a wider sense consists of proteins and fluorides.

Bicarbonate buffer is the dominant buffer during stimulated salivary secretion and represents a combination of bicarbonate and carbonic acids. Its concentration in unstimulated saliva is 1 mM, while the concentration of that buffer reaches a value of 60 mM when salivation is stimulated.

Phosphate buffer is the dominant buffer of unstimulated saliva. Buffer represents a combination of primary and secondary phosphate, whose concentration in unstimulated saliva is 7-8 mM, while during stimulated salivation, this value decreases to 2-3 mM.

Both buffers have a significant effect on acidification reduction in the mouth, thus helping to prevent saliva reduction in the mouth [5].

2. AIM

1. To determine the minimum and maximum value of stimulated and unstimulated saliva in the patient and control group.

2. Determine the unstimulated and stimulated saliva buffering capacity in the patient and control group.

3. Determine the buffering capacity mean value in relation gender in the patient and control group.

3. MATERIAL AND METHOD

In this research, the respondents were divided into two groups, the patient group, which includes respondents who have been on antihypertensive therapy for five years and more and the control group (healthy respondents). After the anamnesis and a detailed extraoral and intraoral examination, the respondent is seated in a chair in a passive sitting position with the head slightly tilted forward, arms and shoulders relaxed.

Laboratory research was conducted by taking a sample of unstimulated and stimulated saliva of the respondent before breakfast, without previously taking food or drink, and before oral hygiene activities, between 6:30 and 7:30 AM, by spitting out the amount of saliva into a sterile plastic cup with a lid, which is marked with a number (1-31) and the letter N – unstimulated. After that, the respondent is given a paraffin ball, which they put in their mouth and chew it for 5 minutes, in that way they stimulate the saliva secretion and spit out a volume of saliva during the time of 5 minutes. The obtained samples are marked with the letter S- stimulated for each respondent separately, and immediately transported to the laboratory. During transport, the samples are stored in a mobile refrigerator at a temperature of 4°C until analysis. 10 minutes later, the samples are used for analysis. It is very important that the sam-

ples are not frozen, but are used for analysis immediately after they are obtained.

The resulting saliva sample is poured into a graduated cylinder, so that the measured saliva amount is recorded and divided by 5, in order to obtain the value per milliliter of saliva per minute, and the final value obtained is recorded in the prepared questionnaire. Normal saliva secretion is about 0.5ml/min.

The measured amount of saliva is used to determine the pH value. Saliva pH values are determined with a pH meter (HANNA instruments 8521). The obtained amount of saliva is diluted with distilled water. Each sample is divided into two equal parts, and its pH value is measured by inserting the pH meter probe into the cup with the saliva sample, and after a certain time, the pH saliva value is read on the pH meter monitor.

After that, the saliva is titrated with an acid (HCl) and a base (NaOH), by adding 100µl of HCl to each sample and 100µl of NaOH to each sample, ranging from pH 3 to pH 11. Each of the measured values is recorder after inserting the micropipette into the ring container, and the pH value for each concentration is displayed on the monitor individually.

4. RESEARCH RESULTS

Results of buffering capacity in the patient and control groups after titration with HCl and NaOH

Buffering capacity is the saliva "resistance" to change pH when treated with acid or base. In other words, the saliva to which more acid or base needs to be added, in order for its pH to decrease or increase by a certain value, has a greater buffering capacity.

Table 1. *Maximum and minimum pH value in unstimulated and stimulated saliva in the patient group*

Max	4.14	3.78
Min	0.38	0.56

The maximum pH value is 4.14 expressed in unstimulated saliva with male respondents, which means that its buffering capacity is the highest because it is necessary to consume the largest amount of HCl to change value by one measuring point. The lowest pH value is 0.38 in unstimulated saliva with male respondents, which means that a minimal amount of HCl is needed to change the pH by one measuring point.

The maximum value in stimulated saliva is measured with male respondents and is 3.78, while the minimum value in stimulated saliva with male respondents is 0.56.

Table 2. *Maximum and minimum pH value in unstimulated and stimulated saliva in the control group*

Max	5.28	5.82
Min	1.88	1.80

The maximum pH value is 5.28 expressed in unstimulated saliva with female respondents, which means that its buffering capacity is the highest because it is necessary to consume the largest amount of HCl in order to change the value by one measuring point. The lowest pH value is 1.88 in unstimulated saliva with female respondents, which means that, in order to change the pH value by one measuring point in that saliva, a minimum amount of HCL is needed. The maximum value in stimulated saliva is measured with male respondents and it is 5.82, while the minimum value in stimulated saliva with female respondents is 1.80.

Table 3. *The average value with the patient group in unstimulated and stimulated saliva treated with HCl*

Patients unstimulated – stimulated		
t-Test: two samples assuming unequal variances		
	Patients (unstimulated-U)	Patients (stimulated-S)
Average value	**1.514555374**	**1.572446256**
Variance	0.854322192	0.710747556
Number of samples	31	31
Hypothetical average value difference	0	
Leeway	59	
t Stat	0.257646448	
P(T<=t) unilaterally	0.398788154	
t Critical unilaterally	1.671093033	
P(T<=t) bilaterally	**0.797576308**	
t Critical bilaterally	2.000995361	

By testing statistically significant difference between the average pH value in unstimulated and stimulated saliva, with the patient group, it was determined that there is no statistically significant difference. Value t=0.797576308. The difference between the average pH values is not statistically significant (t<0.05). However, there is the average value difference between these two groups.

***Table 4**. the average pH value with control group in unstimulated and stimulated saliva treated with HCl*

Control unstimulated – stimulated		
t-Test: two samples assuming unequal variances		
	Control (unstimulated-U)	Control (stimulated-S)
Average value	**3.294714385**	**3.112455638**
Variance	0.688940032	0.724673126
Number of samples	31	31
Hypothetical average value difference	0	
Leeway	60	
t Stat	0.85350081	
P(T<=t) unilaterally	0.198388137	
t Critical unilaterally	1.670648865	
P(T<=t) bilaterally	**0.396776274**	
t Critical bilaterally	2.000297804	

By testing statistically significant difference between the average pH value in unstimulated and stimulated saliva, with the control group, it was determined that there is no statistically significant difference. Value t=0.396776274. The difference between the average pH values is not statistically significant (t<0.05).

***Table 5.** The average pH value in unstimulated saliva with patients and control groups treated with HCl*

Unstimulated patients – control group		
t-Test: two samples assuming unequal variances		
	Patients (unstimulated-U)	Control (unstimulated-U)
Average value	**1.514555374**	**3.294714385**
Variance	0.854322192	0.688940032
Number of samples	31	31
Hypothetical average value difference	0	
Leeway	59	
t Stat	-7.978473026	
P(T<=t) unilaterally	2.97514E-11	
t Critical unilaterally	1.671093033	
P(T<=t) bilaterally	**0.000000000060**	
t Critical bilaterally	2.000995361	

By testing statistically significant difference between the average pH value in unstimulated saliva, with the patient and control group, it was determined that there is a statistically significant difference. Value t=0.000000000060. The difference between the average pH values is statistically significant (t<0.05).

***Table 6.** The average pH value in stimulated saliva with patients and control group treated with HCl*

Stimulated patients – control		
t-Test: two samples assuming unequal variances		
	Patients (stimulated-S)	Control (stimulated-S)
Average value	**1.572446256**	**3.112455638**
Variance	0.710747556	0.724673126

Number of samples	31	31
Hypothetical average value difference	0	
Leeway	60	
t Stat	-7.15672969	
P(T<=t) unilaterally	6.75665E-10	
t Critical unilaterally	1.670648865	
P(T<=t) bilaterally	**0.000000001351**	
t Critical bilaterally	2.000297804	

By testing statistically significant difference between the average pH value in stimulated saliva, with the patient and control group, it was determined that there is a statistically significant difference. Value t=0.000000001351. The difference between the average pH values is statistically significant (t<0.05).

Table 7. *The average pH value with the patient group in unstimulated and stimulated saliva treated with NaOH*

Patients unstimulated – stimulated (NaOH)		
t-Test: two samples assuming unequal variances		
	Patients (unstimulated-U)	Patients (stimulated-S)
Average value	2.898708612	3.22181354
Variance	9.898423226	7.746932602
Number of samples	31	31
Hypothetical average value difference	0	
Leeway	59	
t Stat	-0.428261686	
P(T<=t) unilaterally	0.335010002	
t Critical unilaterally	1.671093033	
P(T<=t) bilaterally	0.670020004	
t Critical bilaterally	2.000995361	

By testing statistically significant difference between the average pH value in unstimulated and stimulated saliva, with the patient group, it was determined that there is no statistically significant different. Value t=0.670020004. The difference between the average pH values is not statistically significant (t<0.05). However, the average pH value with stimulated saliva is larger in comparison with unstimulated saliva (3.22-2.90).

Table 8. *The average pH value with control group in unstimulated and stimulated saliva treated with NaOH*

Control unstimulated – stimulated		
t-Test: two samples assuming unequal variances		
	Control (unstimulated-U)	Control (stimulated-S)
Average value	3.828039679	4.174060168
Variance	1.838060719	3.031037909
Number of samples	31	31
Hypothetical average value difference	0	
Leeway	57	
t Stat	-0.873088719	
P(T<=t) unilaterally	0.193139039	
t Critical unilaterally	1.672028889	
P(T<=t) bilaterally	0.386278078	
t Critical bilaterally	2.002465444	

By testing statistically significant difference between the average pH value in unstimulated and stimulated saliva, with the control group, it was determined that there is no statistically significant difference. Value t=0.386278078. The difference between the average pH values is not statistically significant (t<0.05). However, the average pH value with stimulated saliva is larger in comparison with unstimulated saliva (4.17-3.83).

Table 9. *the average pH value in unstimulated saliva with patients and control group treated with NaOH*

Unstimulated patients – control		
t-Test: two samples assuming unequal variances		
	Patients (unstimulated-U)	Control (unstimulated-U)
Average value	2.898708612	3.828039679
Variance	9.898423226	1.838060719
Number of samples	31	31
Hypothetical average value difference	0	
Leeway	41	
t Stat	-1.510366339	
P(T<=t) unilaterally	0.069309623	
t Critical unilaterally	1.682878003	
P(T<=t) bilaterally	0.138619247	
t Critical bilaterally	2.019540948	

Table 10. *The average pH value in stimulated saliva with patients and control group treated with NaOH*

Stimulated patients – control		
t-Test: two samples assuming unequal variances		
	Patients (stimulated-S)	Control (stimulated-S)
Average value	3.221813542	4.174060168
Variance	7.746932602	3.031037909
Number of samples	31	31
Hypothetical average value difference	0	
Leeway	50	
t Stat	-1.614960088	
P(T<=t) unilaterally	0.05630649	
t Critical unilaterally	1.675905026	
P(T<=t) bilaterally	0.112612979	
t Critical bilaterally	2.008559072	

By testing statistically significant difference between the average pH value in unstimulated saliva with the control and patient group, it was determined that there is no statistically significant difference. Value t=0.138619247. The difference between the average pH values is not statistically significant (t<0.05). However, the average pH value with the control group is larger in comparison with the patient group (3.83-2.90).

By testing statistically significant difference between the average pH value in stimulated saliva, with the control and patient group, it was determined that there is no statistically significant difference. Value t=0.112612979. The difference between the average pH values is not statistically significant (t<0.05). However, the average pH value with the control group is larger in comparison with the patient group (4.17-3.22).

Table 11. The average pH value with the patient group in unstimulated saliva with male and female respondents treated with HCl

Patients unstimulated (U)		
t-Test: two samples assuming unequal variances		
	Female (unstimulated-U)	Male (unstimulated-U)
Average value	1.609501	1.445983
Variance	0.54337869	1.1121937
Number of samples	13	18
Hypothetical average value difference	0	
Leeway	29	
t Stat	0.50805715	
P(T<=t) unilaterally	0.3076281	
t Critical unilaterally	1.699127	
P(T<=t) bilaterally	0.6152562	
t Critical bilaterally	2.04522961	

By testing statistically significant difference between the average pH value in different gender groups, with the patient group in unstimulated saliva, it was determined that there is no statistically significant different. Value t=0.6152562. The difference between the average pH values is not statistically significant (t<0.05). However, the average pH value with female respondents is larger in comparison with male respondents (1.61-1.45).

Table 12. *The average pH value with the patient group in stimulated saliva with male and female respondents treated with HCl*

Patients – stimulated (S)		
t-Test: two samples assuming unequal variances		
	Female (S)	Male (S)
Average value	1.707692	1.474769
Variance	0.69976659	0.736218
Number of samples	13	18
Hypothetical average value difference	0	
Leeway	26	
t Stat	0.75677945	
P(T<=t) unilaterally	0.22799275	
t Critical unilaterally	1.7056179	
P(T<=t) bilaterally	0.4559855	
t Critical bilaterally	2.05552942	

By testing statistically significant difference between the average pH value in stimulated saliva, with the patient group, it was determined that there is a statistically significant difference. Value t=0.4559855. The difference between the average pH values is statistically significant (t<0.05).

Table 13. *The average pH value with control group in unstimulated saliva with male and female respondents treated with HCl*

Control unstimulated (U)		
t-Test: two samples assuming unequal variances		
	Female (U)	Male (U)
Average value	3.14811864	3.4976931
Variance	0.70863579	0.6415802
Number of samples	18	13
Hypothetical average value difference	0	
Leeway	27	
t Stat	-1.1736174	
P(T<=t) unilaterally	0.12539874	
t Critical unilaterally	1.70328842	
P(T<=t) bilaterally	0.25079748	
t Critical bilaterally	2.05183049	

By testing statistically significant difference between the average pH value in unstimulated saliva, with the control group, it was determined that there is a statistically significant difference. Value t=0.25079748. The difference between the average pH values is statistically significant (t<0.05).

Table 14. *The average pH value with control group in stimulated saliva with male and female respondents treated with HCl*

Control (stimulated -S)		
t-Test: two samples assuming unequal variances		
	Female (stimulated-S)	Male (stimulated-S)
Average value	2.77766252	3.5760153
Variance	0.56138484	0.6154631
Number of samples	18	13
Hypothetical average value difference	0	
Leeway	25	
t Stat	-2.8488745	
P(T<=t) unilaterally	0.00432735	
t Critical unilaterally	1.70814075	
P(T<=t) bilaterally	0.0086547	
t Critical bilaterally	2.05953854	

By testing statistically significant difference between the average pH value in stimulated saliva, with the control group, it was determined that there is a statistically significant difference. Value t=0.0086547. The difference between the average pH values is statistically significant (t<0.05).

Table 15. *The average pH value with the patient group in unstimulated saliva with female and male respondents treated with NaOH*

Patients unstimulated (U)		
t-Test: two samples assuming unequal variances		
	Female (U)	Male (U)
Average value	**2.37572809**	**3.2764168**
Variance	3.54655966	14.604143
Number of samples	13	18
Hypothetical average value difference	0	
Leeway	26	
t Stat	-0.8650263	
P(T<=t) unilaterally	0.19746791	
t Critical unilaterally	1.7056179	
P(T<=t) bilaterally	0.39493583	
t Critical bilaterally	2.05552942	

By testing statistically significant difference between the average pH value in unstimulated saliva, with the patient group, it was determined that there is no statistically significant difference. Value t=0.39493583. The difference between the average pH values is statistically significant (t<0.05). However, the average pH value with male respondents is larger in comparison with female respondents (3.28-2.38).

Table 16. *The average pH value with the patient group in stimulated saliva with male and female respondents treated with NaOH*

Patients stimulated (S)		
t-Test: two samples assuming unequal variances		
	Female	Male
Average value	**2.90272638**	**3.4522654**
Variance	8.0514181	7.8536118
Number of samples	13	18
Hypothetical average value difference	0	
Leeway	26	
t Stat	-0.5348577	
P(T<=t) unilaterally	0.29864665	
t Critical unilaterally	1.7056179	
P(T<=t) bilaterally	0.5972933	
t Critical bilaterally	2.05552942	

By testing statistically significant difference between the average pH value in stimulated saliva, with the patient group, it was determined that there is no statistically significant difference. Value t=0.5972933. The difference between the average pH values is not statistically significant (t<0.05). However, the average pH value with male respondents is larger in comparison with female respondents (3.45-2.90).

Table 17, *The average pH value with control group in unstimulated saliva with male and female respondents treated with NaOH*

Control unstimulated (U)		
t-Test: two samples assuming unequal variances		
	Female	Male
Average value	**3.49991287**	**4.2823691**
Variance	2.08330693	1.258683
Number of samples	18	13
Hypothetical average value difference	0	
Leeway	29	
t Stat	-1.6971421	
P(T<=t) unilaterally	0.05018938	
t Critical unilaterally	1.699127	
P(T<=t) bilaterally	0.10037877	
t Critical bilaterally	2.04522961	

Table 18. *The average pH value with control group in stimulated saliva with male and female respondents treated with NaOH*

Control stimulated (S)		
t-Test: two samples assuming unequal variances		
	Female	Male
Average value	**3.77551031**	**4.7258984**
Variance	3.47197307	2.0908007
Number of samples	18	13
Hypothetical average value difference	0	
Leeway	29	
t Stat	-1.5979835	
P(T<=t) unilaterally	0.06044353	
t Critical unilaterally	1.699127	
P(T<=t) bilaterally	0.12088707	
t Critical bilaterally	2.04522961	

By testing statistically significant difference between the average pH value in unstimulated saliva, with the control group, it was determined that there is no statistically significant difference. Value t=0.10037877. The difference between the average pH values is not statistically significant (t<0.05). However, the average pH value with male respondents is larger in comparison with female respondents (4.28-3.50).

By testing statistically significant difference between the average pH value in stimulated saliva, with the patient group, it was determined that there is no statistically significant difference. Value t=0.12088707. The difference between the average pH values is not statistically significant (t<0.05). However, the average pH value with male respondents is larger in comparison with female respondents (4.75-3.78).

5. DISCUSSION

In this research, there were two groups, one of which was represented by respondents who have been on antihypertensive therapy for five years or more, while the other group was represented by respondents who are relatively health and have not used medicines. The age in both groups was similar. The average age with the patient group was 62.2 years, while the average age with the control group was 58.5 years. It can be determined that there was no statistically significant difference between these two groups when it comes to age. As far as gender is concerned, both female and male population were roughly equally present. In the patient group the percentage was 42% women, and 58% men, while in the control group there were 58% women and 42% men. There was no statistical significance in this division either.

For decades, dentists have measured the saliva's buffering capacity and the amount of bacteria in order to estimate the risk of tooth damage [6]. The saliva flow, buffering capacity and saliva's content represent very important factors for oral health [7]. Buffering saliva systems are responsible for maintaining proper acid-bas balance. In 1959, Ericsson developed buffering capacity laboratory by measuring pH, which is divided into three categories (high, medium, and low). Kitasako modified his study by adding HCl in different quantities, depending on the person. However, his formula for calculating the buffering capacity had shortcomings, so Ericsson's formula represents the "golden" standard for salivary buffering capacity measurement. The literature also mentions the tests that are used, namely the modified Ericsson test, colorimetric strip tests, manual pH meter for quantitative pH value determination. The most common variant of collecting stimulated saliva for pH value determination is chewing a paraffin ball for five minutes which was also used in this research [8]. Nowadays, scientific and technological progress in biochemistry, microbiology and immunology lead to the new biomarkers' discovery in saliva, that can be used when detecting systemic diseases as well as ischemic heart disease, cardiac arrest and carcinoma. [9,10,11,12]. This relation between oral and general health has led to renewed interest in the use of saliva as a diagnostic fluid. Saliva gives several privileges compared to traditional biochemical blood analysis. Saliva collection is non-invasive and not stressful for the person taking the sample. Saliva also has a minimal risk for infectious disease transmission such as HPV, HCV and HIV. Saliva is also an ideal biofluid for developing countries, considering the low costs required for sample collection and processing.

In this research, the average amount of unstimulated saliva in the patient group was 1.739ml/5min, and in the control group it was 3.535ml/5min. There is a statistically significant different in the amount of secreted unstimulated saliva t=0.000042, i.e., the respondents in the control group excrete significantly greater amount of saliva. Stimulated saliva average amount in the patient group is smaller and is 3.594ml/5min compared to the control group where it is 6.271ml/5min. There is a statistical significance t=0.000231. These results are consistent with those of Leandro Faria de Matos who compared the patent with the control group [13]. Kagawa et al. have obtained slightly different results in which, with the patient and control group, there is no difference in the amount of secreted unstimulated and stimulated saliva, in comparison with Arauja's research [14,15]. Nauntofte showed in his research that patients had xerostomia increasing by the period of treatment with diuretics compared with the healthy respondents, which is in agreement with this research [16]. The reduced amount of saliva in the patient group was shown in both unstimulated and stimulated saliva, which is obviously a reaction to the long-term antihypertensive medicine use, and this is confirmed by Marton and Murray in their research [17, 18]. In his work, Toshimi showed that by applying calcium channel blockers and their mechanism, pressure is exerted on the water secretion of hard dental tissues and consequently cause a decrease in saliva secretion [19].

Fenoll-Palomares showed in his work that the amount of saliva and the buffering capacity value is higher with male respondents then in female, which is partially in agreement with the results of this research [20]. In this research, the control group has average value of buffering capacity in unstimulated saliva higher with male respondents and is 3.50 in comparison with female, where the average value is 3.15. There is no statistically significant difference between the average buffering capacity values. However, there is the average buffering capacity difference in favor of male respondents, which explains why male respondents need a larger amount of buffer needs to be used in order to change the pH value by one measurement point. In stimulated saliva in the

control group, the buffering capacity was shown to be significantly lower with female respondents 2.78, compared with male where it is 3.58. The average buffering capacity value is statistically significantly different in stimulated saliva in the control group, t=0.008. Stimulated saliva showed a higher buffering capacity in both the patient and control group, compared to unstimulated saliva in both groups, i.e., stimulated saliva is more resistant to pH changes caused by HCl titration. This result is in agreement with Moritsuka's research [21]. In the control group in unstimulated saliva, the average buffering capacity value is 7.842, and in the patient group it is 7.561. There is no statistical significance in unstimulated saliva, however, the average value in the control group is higher than in the patient group. In stimulated saliva, the average value in the control group is also higher 8.066 in comparison with the patient group where it is 7.628. In stimulated saliva, there is a statistically significant difference between the buffering capacity average value, t=0.0060. A lower pH value in the patient group as well as a smaller amount of saliva secreted implies that the patients' saliva "leans" more towards acidity than the control group's saliva, which corresponds to this research. Such patients with a reduced amount of saliva have a much higher risk of developing dental erosions. The reduced pH value and reduced amount of saliva can be explained by the direct influence of antihypertensive medicine activity mechanism on the saliva secretion stimulation. Calcium channel blockers (Ca antagonists) put pressure on water secretion from calcium channels by blocking them, and consequently cause dry mouth [22]. Xerostomia causes increased acidity in the mouth, which is dependent on the amount of saliva and salivation [23]. Antihypertensive medicine activity mechanism leads to a decrease in pH value, which consequently leads to hard dental tissue demineralization.

6. CONCLUSION

The initial pH value in unstimulated and stimulated saliva is higher in the control group in comparison with the patient group. The pH value, as well as the buffering capacity, is higher with male respondents than in female. In this research, it was shown that the saliva "resistance" is higher in unstimulated compared with stimulated saliva, and with male respondents.

7. LITERATURE

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LABORATORIJSKO ISPITIVANJE PUFERSKOG KAPACITETA NESTIMULISANE I STIMULISANE PLJUVAČKE KOD EKSPERIMENTALNE I KONTROLNE GRUPE NAKON TITRACIJE S HCl I NaOH

Sažetak: Uvod: Kapacitet pufera je „otpor“ pljuvačke da promijeni pH kada se tretira kiselinom ili bazom. Drugim riječima, pljuvačka ima veći puferski kapacitet kome treba dodati više kiseline ili baze da bi se njen pH smanjio ili povećao za određenu vrijednost.
Cilj: Laboratorijsko ispitivanje puferskog kapaciteta nestimulisane i stimulisane pljuvačke kod pacijenata eksperimentalne grupe i ispitanika kontrolne grupe u odnosu na uzrast i pol.
Materijal i metoda: Određivanje pH vrednosti pljuvačke vrši se pH metrom (HANNA instruments 8521). Dobijena količina pljuvačke se razblaži destilovanom vodom. Svaki uzorak se dijeli na dva jednaka dijela i mjeri se pH vrednost uzorka. Titracija pljuvačke se vrši kiselinom (HCl) i bazom (NaOH), dodajući po 100 µl HCl u svaki uzorak i 100 µl NaOH u svaki uzorak, u opsegu od pH 3 do pH 11.
Rezultati: Ne postoji statistička značajnost razlika u srednjim vrijednostima kapaciteta pufera.
Zaključak: Postoji razlika u srednjim vrijednostima puferskog kapaciteta u korist muškaraca, što objašnjava da je kod muškaraca potrebna veća količina potrošenog pufera da bi se pH promjenio za jednu jedinicu.

Ključne reči: stimulisana pljuvačka, nestimulisana pljuvačka, kapacitet pufera, pH vrijednost.

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