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Professional paper

DETERMINATION OF THE pH VALUE OF UNSTIMULATED AND STIMULATED SALIVA IN A GROUP OF PATIENTS WITH AND WITHOUT DENTAL EROSION

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Abstract

Saliva is the most important biological factor for the prevention of dental erosion. It begins to act even before the acid attack, by increasing the salivary flow rate in response to acidic stimuli. The actual acidity (pH) of the oral environment directly depends on the presence of mixed saliva. Knowing the physiological flow of saliva by measuring the pH of mixed saliva, it is proven that there is a wide range of these values, from the most acidic pH = 5.3 to the alkaline pH = 7.8. This study aims to determine the quantity of stimulated and unstimulated saliva and the pH value of saliva, and to correlate the obtained results between the control group without erosions and the examined group with erosive lesions of the teeth. To achieve the set goal, our research included 60 subjects of both sexes aged 18-40 years. For this purpose, we collected unstimulated and stimulated saliva using the spitting method. The pH value of saliva was determined using a potentiometric method with a pH meter. The results obtained in our study on the effect of reduced saliva pH show an irreversible loss of hard dental substance, which manifests itself as tooth erosion, which confirms our results. The data suggest that salivary stimulation is an effective method to enhance protective oral properties and that individuals in the experimental group may be more prone to an acidic oral environment. Although no significant differences in saliva volume were found between the groups, the pH difference in unstimulated saliva is both statistically and clinically relevant. The experimental group exhibits significantly lower pH in unstimulated saliva, indicating a more acidic oral environment, which may predispose to dental erosion and other acid-related conditions.

Keywords: pH, Saliva, Tooth erosion, Potentiometry

1. Introduction

Saliva is the most important biological factor in preventing dental erosion. It begins to act even before an acid attack, by increasing the salivary flow rate in response to acidic stimuli. This function of saliva creates a favourable scenario for saliva to effectively dilute and clean the acids that come into contact with the tooth surfaces during erosive attacks. The term oral haemostasis refers to a set of mechanisms that participate in the maintenance and preservation of all oral structures, or, more simply put, the preservation of the health of the teeth and oral mucosa. The function of self-cleaning of the oral cavity is carried out with the help of the main component of saliva. The protection of tooth enamel from attrition is achieved with the help of glycoproteins present in saliva, which create an organic deposit on the surface of the tooth enamel - the acquired dental pellicle. The dental pellicle has a lubricating (lubricating) effect, which partially reduces the loss of tooth enamel during the act of mastication. The buffering role of saliva is achieved with the help of numerous salivary buffers. They enable the maintenance of the buffering capacity of saliva (Lee H. et.al, 2012; Edgar, M., Dawes, et.al. 2012).

Saliva is also important for protecting tooth enamel from the unwanted process of demineralization (Lee H. et.al, 2012). It enables the opposite process, which is called remineralization of the damaged surface layers of tooth enamel. Of the components of saliva that participate in the remineralization processes, the most important are calcium and phosphate

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ions. Namely, salivary secretion is saturated with these ions, which are also the main components of the hydroxylapatite crystals of tooth enamel. The average daily volume of saliva is 500-1000 ml. The largest volume of saliva is produced before, during, and after breakfast, reaching its maximum level around 12 o'clock and falling significantly during the night, during sleep. Some physiological and pathological conditions can alter saliva production quantitatively, for example, smell and stimulation, chewing, psychological, hormonal status, tablets, age, heredity, oral hygiene, and physical exercise (Lingström, et.al. 2003; Kaufman, et.al., 2002)

Good salivary flow helps to keep the salivary glands healthy, reduces the risk of infection and calcification of the salivary glands, and, of course, the most important effect of salivary stimulation - protection of the teeth from the erosive effect of acidic beverages and gastric reflux due to the diluting and neutralizing effect of saliva (Schewtzl, 1996). The age as a factor in the secretion of stimulated and unstimulated saliva has no effect, but the effect of drugs such as antihistamines, antihypertensives, antidepressants, antipsychotics, sedatives, diuretics that reduce saliva secretion has a great influence (Edgar, et.al.,2012). The stimulation of the parotid and submandibular salivary glands in adult patients up to 40 years of age, and the results obtained indicate the following values: the parotid salivary gland secretes a minimum of 0.7 ml/min per day (Baliga Sh., 2013), and the submandibular glands a minimum of 0.6 ml/min of stimulated saliva (Krishnamurthy, et.al. 2015). So, the greatest contribution to the secretion of stimulated saliva is made by the parotid gland, which is the largest in volume and has a large capacity for the production of stimulated saliva.

There are several ways to stimulate saliva, and one of them is mechanical stimulation, for which (Doods, 2015), in unstimulated saliva, 0.3 ml/min is secreted, and with chewing paraffin balls, stimulated saliva is secreted daily about 1-2 ml/min. Patients with erosive lesions (experimental group) and without erosive lesions (control) group, and the results of the studies indicate that the amount of unstimulated and stimulated saliva flow in the experimental and control groups is equal. An unstimulated flow rate of whole saliva less than 0.1 ml/min and a stimulated saliva flow rate less than 0.7 ml/min are considered as hypofunction of the salivary glands, and any reduced salivary flow rate increases the risk of erosive lesions of dental structures. (Sivakumar, et.al. 2024; Ericsson, 2014).

In the neutral medium, pH=7.3. Buffer solutions are solutions that maintain an approximately constant pH when small amounts of acids or bases are added or when the solution is diluted. In other words, buffers are resistant to changes in pH, to reaching the pH 7.3 value is very important for many biochemical processes in living organisms (Samet, et.al., 2016). The pH value where the results indicate that when the pH is higher than pH = 6 then the saliva is saturated with phosphate ions, but when the pH value falls below pH = 5.5 then the hydroxylapatite from the tooth enamel is loosened and the teeth are susceptible to erosion. Lennander, examined the critical pH value in patients with erosions and in his results obtained a level of pH = 5.2-5.5, which is considered to be critical when tooth demineralization occurs. With increased secretion of stimulated saliva, the level of proline-rich proteins increases, and these proteins bind to the surface of the tooth enamel and form a dental pellicle that protects the tooth from excessive friction (Deepa, et al. 2014; Lennander, at.al, 2000; Adhani, et.al.,2015).

The modern way of life in the last 30 years has influenced the increase in the prevalence of erosive lesions of the teeth, which today reaches up to 75%. Several possible factors are important in the creation of erosive lesions of the teeth, which are determined by measuring salivary parameters. Considering the multifactorial causes of the growth of erosive lesions in the population, their clinical examination, from our own preliminary examinations, as well as knowledge from the world literature, the purpose of our research emerged, in which we will determine the following:

- To determine the quantity and pH value of secreted unstimulated and stimulated saliva in the studied group of patients;
- To determine the quantity and pH value of secreted unstimulated and stimulated saliva in the control group

2. Materials and Methods

To achieve the set goal, a cross-sectional study including 60 patients of both sexes, aged 18 to 40 years, was conducted at the University Clinical Stomatology Center "Prof. Dr. Bojo Andreski" in Skopje. The biochemical analyses were performed at the Institute of Medical and Experimental Biochemistry, Faculty of Medicine, in Skopje. The sample was divided into two groups the first group consisted of 30 subjects with erosive lesions on the teeth, the experimental group, and the second group consisted of 30 subjects without erosive lesions on the teeth, the control group. **Exclusion criteria for the study were as follows:**

- Presence of chronic diseases (e.g., diabetes, blood dyscrasias, hormonal disorders, hypertension, kidney disease, Sjögren's syndrome, or history of surgical intervention on the salivary glands);
- Certain physiological conditions (e.g., pregnancy or breastfeeding).

For all subjects in both the control and experimental groups, saliva was collected according to the recommendations of (Navazesh, 1993), which are as follows:

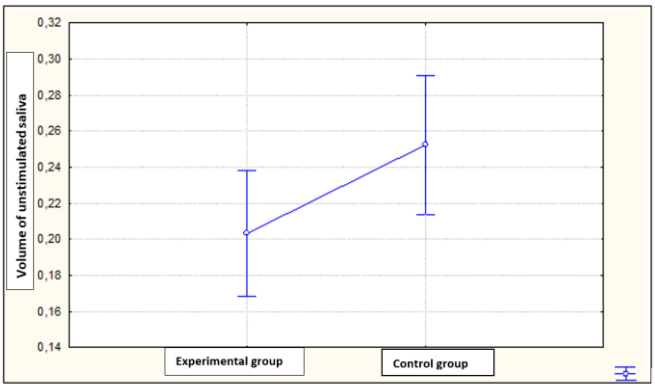
- Saliva samples should be collected at the same time of day, preferably between 9:00 and 11:00 AM.
- Patients should refrain from eating or drinking for at least 90 minutes before collection.
- The mouth should be rinsed with deionized water.
- The subject should sit comfortably, with eyes open and head tilted slightly forward.

Unstimulated saliva collection was performed using the spitting method. Two millilitres of saliva were collected in a graduated tube with a funnel to facilitate collection. The patient was instructed to spit when feeling the urge to swallow the saliva accumulated on the floor of the mouth, spitting every 2 minutes over 10 minutes. **Stimulated saliva collection** was also performed using the spitting method, but with gustatory stimulation. The subject chewed paraffin balls for 5 minutes and spat into graduated tubes as saliva accumulated. The level of secreted mixed saliva was marked on the tube. The volume of saliva was measured in millilitres per minute, providing the average amount of saliva secreted per minute.

The pH value of the saliva samples was determined using the potentiometric method with a pH meter (Hanna Instruments, pH Meter 209), suitable for biological samples like saliva. Potentiometry involves measuring the voltage (electrical potential) of a solution to determine its pH. The pH meter compares the potential difference between two electrodes: Measuring electrode (glass electrode) – sensitive to hydrogen ion activity, and Reference electrode – provides a stable voltage for comparison. The difference in voltage between these electrodes corresponds to the pH of the solution. Glass Electrode has a thin, pH-sensitive glass membrane at the tip. When immersed in a solution, hydrogen ions interact with the membrane, creating a potential difference (voltage). The potential changes depending on the concentration of hydrogen ions (i.e., the pH). The Reference Electrode, contains a known and constant concentration of ions. Usually filled with a solution like KCl (potassium chloride). Provides a constant reference voltage. pH Meter Device, Converts the voltage difference (measured in millivolts) into a readable pH value. Uses the Nernst Equation to relate voltage to pH. Calibration, using buffer solutions (commonly pH 4.0, 7.0, and 10.0). Ensures accurate measurements by rinsing electrodes with distilled water, immersing the electrode in the saliva sample, then waiting for the reading to stabilize, and the meter displays the pH value.

3. Results and Discussion

Saliva as a major defence factor in the mouth and a decrease in its flow rate affects oral health (Strahl, et.al.,1990). Oral homeostasis is a set of mechanisms that participate in maintaining the health of all oral structures. The salivary glands produce a secretion, saliva, which is an important tool for oral homeostasis. Saliva is the water of life of the oral cavity. The absence of saliva in the oral environment is a prerequisite for numerous oral diseases. Saliva is a mixture of secretions secreted by three pairs of major salivary glands (mastoid, submandibular, and sublingual), by minor mucous glands, and by gingival fluid.



Graph 1. Display of the average value of the volume of unstimulated saliva in the two groups.

During our study, one of the goals was to determine the amount of saliva secreted, unstimulated and stimulated, in a study group with erosions and a control group without erosions. The presence of a sufficient amount of saliva in the oral cavity is a prerequisite for maintaining the health of all oral structures.

Table 1 and Graph 1 present the descriptive statistics for the volume of unstimulated saliva in two groups, an experimental group and a control group, each consisting of 30 participants. The average volume in the experimental group is 0.2 ml, which is lower than the control group's average of 0.3 ml. This may indicate a potential impact of the experimental condition on reducing saliva production. The minimum values are quite similar between the groups (0.06 ml in the experimental group and 0.05 ml in the control group), suggesting that the lowest saliva outputs are comparable. The maximum values are also close (0.44 ml vs. 0.42 ml), indicating that some participants in both groups had relatively high saliva volumes. The standard deviations (0.093 for the experimental group and 0.103 for the control group) suggest that the variation in saliva volume is slightly greater in the control group, though both groups show a similar range of variability.

Table 1. Presentation of the average value of the volume of unstimulated saliva in the two groups.

	Number	Averege/ml	Minimum/ml	Maximum/ml	Std. Dev
Experimental group	30	0.2	0.06	0.44	0.092971
Control group	30	0.3	0.05	0.42	0.103247

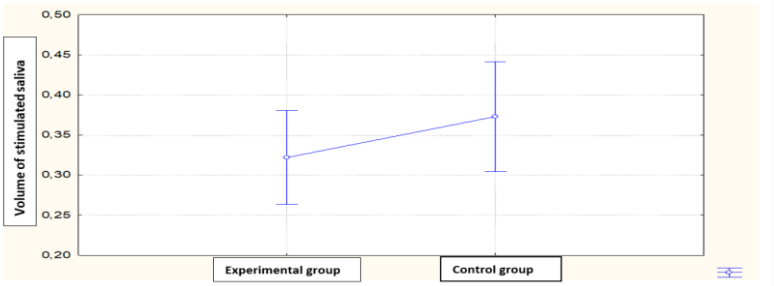
Table 2 shows the results, where, according to the Mann-Whitney U test, the difference between the average values of the volume of unstimulated saliva between the groups with and without erosive lesions is statistically insignificant for $p>0.05$ ($p=0.051$). In our studies, the results of unstimulated saliva in the study group were 0.2-0.09 ml/min, while in the control group it was

0.3-0.1 ml/min (Table 1 and Graph 1), the difference between the average values of unstimulated saliva volume between the groups with and without erosive lesions is statistically insignificant for p>0.05 (Table 2).

Table 2. Display of Mann-Whitney U test

Volume of unstimulated saliva	Rank Sum - group 1	Rank Sum - group 2	U	p-level
	783.5000	1046.500	318.5	0.051

Table 2 presents the results of a Mann-Whitney U test, which was used to compare the volume of unstimulated saliva between the two groups. The experimental group has a total rank sum of 783.5, and Group 2 (Control) has a higher rank sum of 1046.5, suggesting that participants in the control group generally had higher values of unstimulated saliva volume. The difference in unstimulated saliva volume between the experimental and control groups approached statistical significance (U = 318.5, p = 0.051), with the control group showing generally higher saliva volumes. This indicates that there is no statistically significant difference between the two groups at the p > 0.05 level, but the result is very close to significance. It might be described as showing a trend toward significance, suggesting a possible effect that may warrant further investigation with a larger sample size.



Graph 2. Display of the average value of the volume of stimulated saliva in both groups.

Other factors that influence secretion are the size of the salivary glands, where saliva secretion will be determined by the size of the salivary glands. More recent research has shown that aging has very little effect on the secretion of stimulated saliva. It is believed that the decrease in secretion in elderly people is a consequence of taking medications, and not from old age.

Table 3. Presentation of the average value of the volume of stimulated saliva in both groups.

	Number	Average/ml	Minimum/ml	Maximum/ml	Std. Dev
Experimental group	30	0.322	0.12	0.9	0.156765
Control group	30	0.373	0.1	0.7	0.183700

Table 3 and Graph 2 present the descriptive statistics for the volume of stimulated saliva in the experimental and control groups, each consisting of 30 participants. The experimental group had a lower mean volume of 0.322 ml, while the control group had a higher mean of 0.373 ml. This difference suggests that, similar to unstimulated saliva, the experimental condition may be associated with reduced stimulated saliva production. Both groups had a similar minimum (0.12 ml in the experimental group, 0.1 ml in the control group), indicating comparable lower bounds of saliva secretion. The maximum in the experimental group was 0.9 ml, which is notably higher than the control group's maximum of 0.7 ml, indicating a wider range in the experimental group. The experimental group had a standard deviation of 0.1568, while the control group had a

slightly higher variability at 0.1837. This suggests slightly more dispersion in stimulated saliva volumes among participants in the control group.

Table 4. Display of Mann-Whitney U test.

Volume of unstimulated saliva	Rank Sum - group 1	Rank Sum - group 2	U	p-level
	854.5000	975.5000	389.5000	0.371078

Table 4 presents the outcome of a Mann-Whitney U test comparing the volume of stimulated saliva between the two groups, of experimental (group 1=854.5), and control (group 2= 975.5). These rank sums suggest that, overall, the control group had slightly higher ranks, indicating higher saliva volume values compared to the experimental group. The calculated U statistic is 389.5, which is used to determine the degree of overlap between the two groups' distributions. The p-value of 0.371 indicates that the difference in stimulated saliva volume between the groups is not statistically significant ($p > 0.05$). This means we fail to reject the null hypothesis, and there is no strong evidence to suggest a significant difference in stimulated saliva volume between the two groups. "The Mann-Whitney U test showed no statistically significant difference in the volume of stimulated saliva between the experimental and control groups ($U = 389.5$, $p = 0.371$). Although the control group exhibited a slightly higher average volume, this difference was not significant.

The amounts of secreted unstimulated and stimulated saliva in our subjects, both from the control and the study groups, are within the limits of the physiological values of secreted saliva. Although the amount of secreted, unstimulated, and stimulated saliva in subjects without dental erosions was greater compared to the amount of secreted saliva in subjects with dental erosions, this difference was not statistically significant. Our results are in accordance with the literature (Sivakumar, et.al.,2024; Kaufman, et.al., 2002), the secretion of saliva with the stimulation of the parotid and submandibular salivary glands in adult patients up to 40 years of age, the results obtained indicate the following values, the parotid salivary gland secretes a minimum of 0.7 ml/min per day, and the submandibular glands a minimum of 0.6 ml/min of stimulated saliva. So, the parotid gland has the greatest contribution to the secretion of stimulated saliva. There are several ways to stimulate saliva, one of them is mechanical stimulation (Dodds, 2015) in obtained results that in unstimulated saliva 0.3 ml/min of saliva is secreted, and by chewing paraffin balls, stimulated saliva is secreted daily about 1-2 ml/min, and these results are partially in agreement with our results.

These results are expected considering that these are examinees who do not have systemic diseases or conditions, nor do they take medications that could cause reduced saliva secretion. We believe that the absence of statistically significant differences in the amount of saliva secreted between the two groups of examinees indicates that the amount of saliva secreted in our examinees does not affect the occurrence of dental erosion. Therefore, it is more reliable to analyse the constituent components of saliva to determine whether alteration of certain salivary components has an impact on the occurrence of dental erosion.

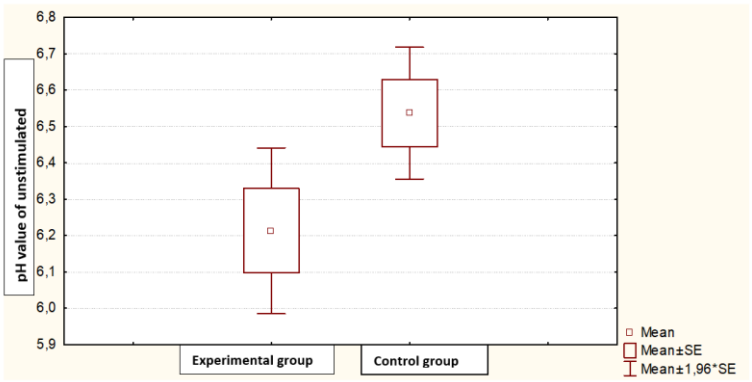
Table 5 displays the results of a Wilcoxon Matched Pairs test (also known as the Wilcoxon signed-rank test) conducted within each group to evaluate whether there is a significant difference between unstimulated and stimulated saliva volumes. The Wilcoxon test is appropriate for comparing paired (related) samples, such as saliva volumes measured in the same individuals under two conditions (unstimulated vs. stimulated). The result is highly statistically significant ($p < 0.001$), indicating a significant difference between unstimulated and stimulated saliva volumes in the experimental group. This is also highly statistically significant ($p < 0.001$), showing a significant difference between the two conditions in the control group as well. Both groups showed a statistically significant increase in saliva volume when stimulated, confirming that the stimulation method used was effective regardless of group

assignment. This result aligns with physiological expectations, as stimulated saliva production is typically higher than unstimulated saliva. The Wilcoxon matched pairs test revealed a statistically significant difference between unstimulated and stimulated saliva volumes in both the experimental group ($T = 50.5$, $Z = 3.61$, $p < 0.001$) and the control group ($T = 48.5$, $Z = 3.65$, $p < 0.001$), indicating that saliva stimulation significantly increased saliva flow in both groups.

Table 5. Display of the Wilcoxon Matched Pairs test.

Volume of unstimulated/stimulated saliva	N	T	Z	p-level
Experimental group	30	50.50000	3.611075	0.000305
Control group	30	48.50000	3.654321	0.000258

The results obtained in this study show that there is a difference between the amount of secreted unstimulated and the amount of stimulated saliva, in the examined and control groups. According to the Wilcoxon Matched Pairs test, this difference is statistically significant for $p<0.05$ (Table 5). This result is not surprising and is in accordance with the generally accepted knowledge and views that with stimulation of various receptors in the oral cavity, in our subjects - mechanoreceptors (paraffin beads) the amount of secreted saliva significantly increases.



Graph 3. Display of the average pH value in unstimulated saliva in both groups.

Knowing the physiological flow of saliva as well as its buffer capacity, it can be said that mixed saliva determines the pH of the oral environment. By measuring mixed saliva, it has been proven that there is a wide range of these values from the most acidic pH = 5.3 to the alkaline one, which is pH = 7.8. Table 6 and Graph 3 presents the descriptive statistics for the pH of unstimulated saliva in both the experimental and control groups, each consisting of 30 participants. This indicates that the control group had a slightly more alkaline (less acidic) unstimulated saliva on average. A lower pH (as in the experimental group) may be associated with higher risk of dental erosion or altered oral conditions. The experimental group had a minimum pH of 5.0, which is notably more acidic than the control group's minimum of 5.5. Both groups share the same maximum pH value of 7.5, which is within the neutral range. The experimental group had a higher standard deviation (0.637) compared to the control group (0.508), indicating greater variability in unstimulated saliva pH values within the experimental group. These data suggest that the experimental group tended to have a more acidic oral environment in unstimulated conditions, which could have implications for oral health (e.g., susceptibility to erosion or dental caries). The lower average pH and greater variation in the experimental group may reflect environmental or physiological influences related to the experimental condition.

Table 6. Presentation of the average pH value in unstimulated saliva in both groups (pH scale range (0–14))

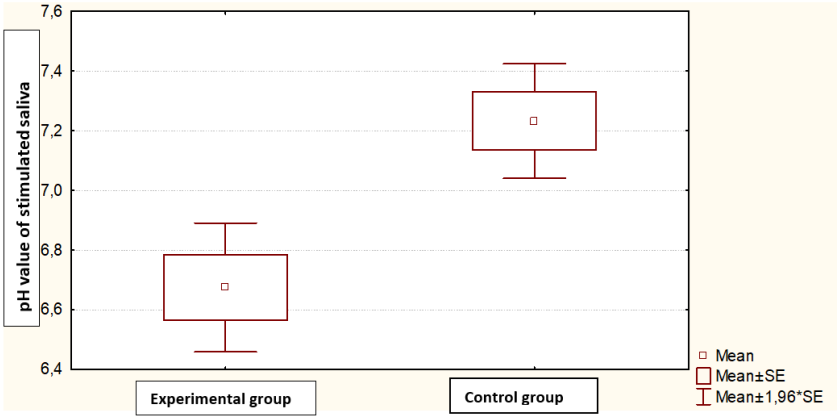
pH of unstimulated saliva	Number	Averege/ml	Minimum/ml	Maximum/ml	Std. Dev
Experimental group	30	6,2	5,0	7,5	0,636658
Control group	30	6,5	5,5	7,5	0,507518

Table 7 presents the results according to the Mann-Whitney U test, and the difference between the average values of pH in unstimulated saliva between the two groups is statistically significant for $p<0.05$ ($p=0.039876$). This result is statistically significant at the $p < 0.05$ level, indicating that the pH of unstimulated saliva is significantly lower in the experimental group compared to the control group. This confirms earlier observations based on descriptive statistics. The study evaluated the volume and pH of unstimulated and stimulated saliva in experimental and control groups to explore differences in salivary characteristics related to dental health. Descriptive data showed that the control group had higher mean volumes of both unstimulated and stimulated saliva, but these differences were not statistically significant (Tables 1–4). Within both groups, Wilcoxon tests showed a significant increase in saliva volume after stimulation ($p < 0.001$, Table 5), confirming that salivary stimulation was effective.

Table 7. Display of Mann-Whitney U test.

pH of unstimulated saliva	Rank Sum – group 1	Rank Sum –group 2	U	Z	p-level
	776.0	1054.0	311.0	-2.05504	0.039876

The experimental group had a significantly lower pH in unstimulated saliva compared to the control group ($U = 311.0$, $p = 0.040$, Table 7). This suggests a more acidic oral environment in the experimental group, which may increase the risk of dental erosion or demineralization. After stimulation, pH values increased in both groups (Table 8), with the control group showing a higher mean stimulated pH (7.2) compared to the experimental group (6.7). The Wilcoxon Matched Pairs Test (Table 10) confirmed that pH significantly increased after stimulation in both groups ($p < 0.001$), with the control group showing a more uniform response ($T = 0.0$).



Graph 4. Display of the average pH value in stimulated saliva in both groups.

The actual acidity (pH) of the oral environment directly depends on the presence of mixed saliva. Knowing the physiological flow of saliva by measuring the pH of mixed saliva, it is proven that there is a wide range of these values, from the most acidic pH = 5.3 to the alkaline pH = 7.8. The obtained pH values of course, depend on the measurement and volume of salivary secretion, i.e., whether it is unstimulated or stimulated secretion. Mixed saliva consists of the following buffers:

Bicarbonate buffer - is the dominant buffer in stimulated salivary secretion. According to its biochemical composition, the bicarbonate buffer is a combination of sodium bicarbonate $NaHCO_3$ and carbonic acid H_2CO_3 in a ratio of (1:20. In unstimulated saliva, its secretion is 1 mmol/l, and in stimulated salivary secretion, the concentration reaches 60 mmol/l.

The significance of this buffer and its increased concentration is multiple:

- The pH values reach pH = 7.8, whereby the mixed saliva becomes slightly alkaline;
- The dissolving property of the main component of saliva - water, which protects tooth substances from the loss of minerals, decreases;
- In alkaline conditions, the remineralization of the surface layers of the tooth is stimulated as a result of the deposition of minerals on the surface of the teeth. This is actually the first significant phase of recrystallization of the tooth surfaces.
- Alkaline saliva, in turn, creates prerequisites for the absorption of salivary proteins on the surface of the teeth during mastication, protecting the teeth from excessive wear.

Phosphate buffer - is a dominant buffer and its concentration in unstimulated saliva is 7-8 mmol/l, while in stimulated saliva it is 2-3 mmol/l.

Table 8 and Graph 4 show the average pH values in stimulated saliva in the study group, which is 6.7 ± 0.6 (acidic environment), with a minimum value of 5.5 and a maximum of 7.5, while in the control group, it is 7.2 ± 0.5 (neutral environment), with a minimum value of 6 and a maximum of 8. This suggests that the control group had more alkaline-stimulated saliva, which could indicate better buffering capacity or oral health conditions. The control group had both a higher minimum and maximum, meaning the pH values were generally higher and more alkaline. Both groups show similar variability, with slightly more variation in the experimental group. These results support the idea that stimulated saliva tends to have a higher pH (more alkaline) compared to unstimulated saliva in both groups. The experimental group consistently shows lower pH values, suggesting a more acidic oral environment, which may be a concern for dental erosion or acidic dietary habits.

Table 8. Presentation of the average value of pH in stimulated saliva in both groups.

	Rank Sum – group 1	Rank Sum –group 2	U	Z	p-level
Experimental group	30	6.7	5.5	7.5	0.602116
Control group	30	7.2	6.0	8.0	0.537127

In Table 9, according to the Mann-Whitney U test, the difference between the average values of pH in stimulated saliva between the two groups with and without erosive lesions is statistically significant for $p<0.05$ ($p=0.001557$). This result is highly statistically significant ($p < 0.01$), indicating a clear difference in the pH of unstimulated saliva between the groups. Specifically, the experimental group has significantly lower pH, i.e., a more acidic oral environment, than the control group. This strengthens the previous conclusion that oral acidity may be more pronounced in the experimental group.

Table 9. Mann-Whitney U test display.

pH of unstimulated saliva	Rank Sum – group 1	Rank Sum - group 2	U	Z	p-level
	701.0	1129.0	236.0	-3.16387	0.001557

In Table 10, the Wilcoxon Matched Pairs test shows that the difference between the average pH values of unstimulated and stimulated saliva in the study group is statistically significant at $p<0.05$ ($p=0.000037$), while in the control group it is also statistically significant at $p<0.05$ ($p=0.000002$). Table 10 shows the results of the Wilcoxon Signed-Rank Test, a non-parametric test used to assess whether there is a statistically significant difference between two related samples—in this case, the pH of unstimulated and stimulated saliva within each group. This result indicates a highly statistically significant increase in pH between unstimulated and stimulated saliva in the experimental group. This result is also highly statistically significant, showing an even more consistent increase in pH in the control group when saliva is stimulated. Both groups experienced a significant rise in saliva pH after stimulation. The control group result ($T = 0.0$) suggests that all participants had a higher pH in stimulated saliva than in unstimulated saliva—this is the strongest possible outcome in a Wilcoxon test. The experimental group also showed a strong but slightly less uniform increase. This supports the general understanding that saliva stimulation increases its pH, likely due to enhanced bicarbonate secretion that improves buffering capacity—an important factor in protecting against dental erosion and acid attacks. The Wilcoxon matched pairs test revealed a statistically significant increase in pH following saliva stimulation in both the experimental ($T = 32.0$, $Z = 4.12$, $p < 0.001$) and control groups ($T = 0.0$, $Z = 4.78$, $p < 0.001$), confirming the effectiveness of stimulation in raising salivary pH.

Table 10. Wilcoxon Matched Pairs Test Results: Comparison of pH in Unstimulated and Stimulated Saliva.

pH of unstimulated/stimulated saliva	N	T	Z	p-level
Experimental group	30	32.0	4.123952	0.000037
Control group	30	0.00	4.782139	0.000002

The study assessed the volume and pH levels of unstimulated and stimulated saliva in both experimental and control groups to understand physiological differences with relevance to oral health. Although the control group consistently showed higher average saliva volumes, the differences between groups were not statistically significant (Tables 1–4). Stimulation significantly increased saliva volume within both groups ($p < 0.001$, Table 5), confirming the effectiveness of stimulation. The experimental group had a significantly lower pH in unstimulated saliva compared to the control group ($U = 236.0$, $Z = -3.16$, $p = 0.0016$, Table 7). This confirms a more acidic oral environment in the experimental group, which is a statistically robust finding. After stimulation, both groups showed an increase in saliva pH (Table 8), with the control group reaching a higher average stimulated pH (7.2) compared to the experimental group (6.7). The Wilcoxon test confirmed that the increase in pH after stimulation was statistically significant in both groups ($p < 0.001$, Table 10), with particularly uniform improvement in the control group. These findings support the view that monitoring and managing saliva pH, especially in unstimulated conditions, is crucial—particularly for individuals in risk groups similar to the experimental population.

In our study, the average pH values obtained in stimulated saliva in the study group were 6.7-0.6, while in the control group they were 7.2-0.5 (Table 8 and Graph 4), according to the Wilcoxon Matched Pairs test, the difference between the average pH values of unstimulated and stimulated saliva in the study and control groups is statistically significant for $p<0.05$ (Table 10). We believe that the obtained results of the pH value in the control and study groups indicate that the reduced pH value in the study group is significant in the occurrence of erosions. Our results are in accordance with the literature data (Deepa, et al. 2014; Lennander, at.al., 2000; Adhani, et.al., 2015, Silva J.G., et.al.2013)) examined the pH value where the results indicate that when the pH is higher than pH = 6 then the saliva was saturated with phosphates, and when the pH value of the saliva decreases below 5.5, conditions are created for the loss of ions from

the hydroxylapatite of the tooth, its increased solubility and the teeth are susceptible to erosion. Also, in accordance with our results, the critical pH value in patients with erosions and in his results he obtained a level of pH = 5.2-5.5, which is considered critical for demineralization of dental substances, so we can conclude that the occurrence of erosive lesions is due to the decrease in the pH value of the saliva, which increases its acidity. In this case, the balance of electrolytes can be shifted towards dissolution and demineralization, and as a result of this process, erosive lesions of the teeth occur. Of the salivary electrolytes, the presence of calcium in saliva is of great importance for the integrity of the hard tooth structure.

The mineral component of human teeth, found in both enamel and dentine, is primarily composed of a highly substituted form of hydroxyapatite (HAP). Unlike pure hydroxyapatite, which has the idealized formula $Ca_{10}(PO_4)_6(OH)_2$, biological tooth mineral is better represented by the more complex formula:

$Ca_{10-x}Na_x(PO_4)_{6-\gamma}(CO_3)_z(OH)_{2-u}F_u$. This formula reflects the calcium deficiency (10–x) and the presence of substitutions in the mineral structure. Common substitutions include Na^+ (sodium) replacing Ca^{2+}, CO_3^{2-} (carbonate) replacing PO_4^{3-} (phosphate), F^- (fluoride) replacing OH^- (hydroxyl), and traces of Mg^{2+} and K^+ ions (Hyun-Su Lee, 2013, Lussi, et.al.,2011). These substitutions disrupt the crystal lattice and increase the solubility of the mineral in acidic environments. Carbonate substitution, in particular, significantly weakens the crystal structure. As a result, biological HAP is more acid-soluble than synthetic hydroxyapatite, and even more so when compared to fluorapatite (FAP), which has the formula $Ca_{10}(PO_4)_6F_2$. Although enamel and dentine have similar mineral compositions, dentine typically contains 5–6% carbonate, compared to only ~3% in enamel. Additionally, dentine crystals are smaller, increasing surface area and making dentine even more susceptible to acid dissolution. The assumption that pH alone determines erosive potential is misleading. A solution can have a low pH but still be non-erosive if it is supersaturated with respect to tooth mineral. For example: The key factor is the degree of saturation of the fluid surrounding the tooth surface. When this fluid is only slightly undersaturated, an initial phase of demineralization may occur, but the local pH rises and ion concentrations increase, forming a saturated boundary layer that prevents further erosion (Silva, et.al., 2013, Lussi A., et.al.2011).)

In vivo, saliva provides limited protection against this erosion by forming a pellicle layer. The pellicle is a protein rich layer that forms on enamel due to salivary action. HA coated with a pellicle layer undergoes demineralization and re-mineralization reactions depending on the ion product. In the chemical reaction, as the pH decreases, the ionic solution in the pellicle layer becomes unsaturated, resulting in a shift to demineralization (Figure 1), or loss of calcium and phosphate ions from the crystal until a solubility equilibrium is reached (Hyun-Su Lee, 2013).

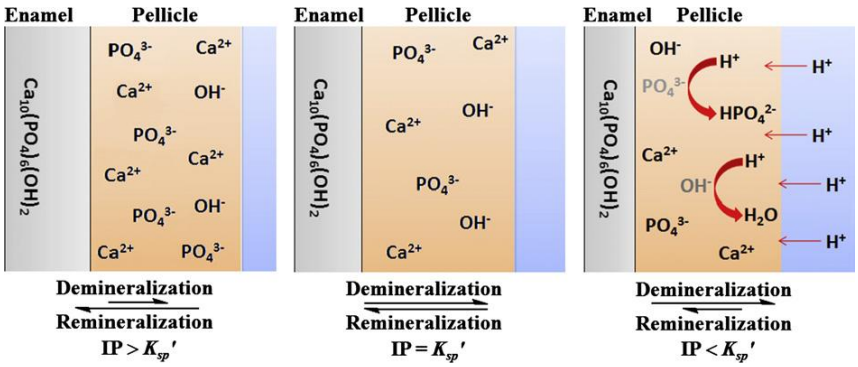


Figure. 1. Chemical reaction conditions corresponding to re-mineralization of enamel, equilibrium between enamel production and degradation, and demineralization of enamel due to acidic exposure (Hyun-Su Lee, 2013).

Because of the big effect of Ca ions present in the saliva on the demineralization and remineralization, our analyses of the calcium ion concentration were conducted. Table 11 presents descriptive statistics of calcium ion concentrations in stimulated saliva for both the experimental and control groups, each consisting of 30 participants. The average calcium ion concentration was 1.1 mmol/L, with values ranging from 0.1 to 1.6 mmol/L. The standard deviation was 0.340291, indicating a relatively higher variability in calcium levels among participants in this group. The average concentration was notably higher at 1.6 mmol/L, with a narrower range from 1.1 to 2.2 mmol/L. The standard deviation was 0.277530, suggesting less variation compared to the experimental group. These results indicate that the control group had both a higher mean calcium ion concentration and a narrower distribution of values. The difference in averages between the two groups may suggest an influence of the variable under investigation.

Table 11. The average value of the calcium ions concentration in stimulated saliva in both groups

	Number	Avarege/mmol/L	Min. /mmol/L	Max./ mmol/L	Std. Dev
Experimental group	30	1.1	0.1	1.6	0.340291
Control group	30	1.6	1.1	2.2	0.277530

Dental enamel is composed primarily of hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, but it also contains several impurities such as carbonate and fluoride. Because the proportions of these impurities are different vary for each person, the solubility is not fixed. When HA is in contact with water, the following reaction occurs:

$$\mathbf{Ca_5(PO_4)_3(OH)\ (solid) + 4\ H^+\ (aq) \rightleftarrows 5Ca^{2+}\ (aq) + 3HPO_4^{2-}\ (aq) + H_2O\ (l)}$$

Solution: a small amount of HA dissolves, releasing calcium, phosphate and hydroxyl ions. This process continues until the water is saturated with respect to HA. At that point, the rate of the forward reaction, mineral dissolution, is equal to the rate of the backward reaction, mineral precipitation(Colin Dawe, 2003). Saliva plays a crucial role in modulating tooth erosion (Gabriel J. et.al., 2013). It acts as a buffer, neutralizing acids and supplying calcium and phosphate ions to re-mineralize early lesions. However, its protective capacity is not unlimited. For instance, studies have shown that consumption of acidic candies significantly decreases oral pH and stimulates salivary flow. Despite this buffering effect, chronic or prolonged exposure to such acids can overcome the natural defences and result in dental erosion, particularly if saliva production is low or compromised.

4. Conclusion

The study aimed to compare the volume and pH of unstimulated and stimulated saliva between experimental and control groups, as well as within each group. The control group had a higher average volume of unstimulated saliva compared to the experimental group. However, this difference approached but did not reach statistical significance (Mann-Whitney U = 318.5, p = 0.051). Similarly, the stimulated saliva volume was slightly higher in the control group (0.373 mL) than in the experimental group (0.322 mL), but again, the difference was not statistically significant (U = 389.5, p = 0.371). Within each group, a Wilcoxon Matched Pairs Test confirmed a statistically significant increase in saliva volume after stimulation ($p < 0.001$ in both groups), indicating that the stimulation procedure was effective. The control group consistently showed slightly higher saliva volumes and more alkaline pH levels than the experimental group, although these differences were not statistically significant in intergroup comparisons.

The pH of unstimulated saliva was lower in the experimental group (mean = 6.2) than in the control group (mean = 6.5), with greater variability observed in the experimental group. This suggests a more acidic oral environment in the experimental group. For stimulated saliva, the average pH increased in both groups, with the experimental group reaching 6.7 and the control group reaching 7.2 (Table 8). This indicates that stimulation raises pH, likely due to increased bicarbonate buffering. The Wilcoxon test results demonstrated a statistically significant increase in pH values after stimulation in both groups. Notably, the control group showed uniform increases in pH across all participants, reflected by a T value of 0.0. However, within both groups, saliva stimulation led to significant increases in both volume and pH, confirming the physiological effect of stimulation on salivary function. The experimental group exhibited a significantly lower pH in unstimulated saliva, pointing to a more acidic oral environment. No significant intergroup differences were observed in saliva volume, although the control group consistently showed higher values. Stimulation significantly increased both saliva volume and pH in both groups, reinforcing its role in enhancing oral protective functions.

These results may suggest that individuals in the experimental group could be more vulnerable to acid-related oral health issues, and that stimulating saliva flow could be a beneficial preventive strategy. Salivary stimulation significantly improves both volume and pH in both groups, highlighting its importance in enhancing oral defense mechanisms. The risk of dental erosion depends not only on pH, but more importantly on the mineral saturation of the surrounding fluid, the presence of protective factors like saliva, and the mineral composition of the teeth. Understanding the chemical structure of tooth minerals—especially the role of carbonate substitution—and how they interact with acidic environments is essential for developing effective strategies to prevent dental erosion.

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