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Protective Role of Salivary Proteins in Dental Erosion: Biochemical Perspectives

Aksu Samet[1]; Akgul Omeragic[1]; Sezen Samet[1]

[1]Faculty of Dental Medicine, International Balkan University Skopje, R. North Macedonia

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Abstract: This study looked at changes in the chemical composition of saliva caused by dental erosion. The areas studied are calcium homeostasis, calcium, total proteins, and some associated factors. This study was done using a cross-sectional design. There were sixty subjects included in the study that were 18 to 40 years old, divided into an experimental group (n=30) with dental erosion and a control group (n=30) without erosive lesions. Saliva samples were collected when no stimulation occurred, and then after stimulation of the salivary glands. Smoking history and frequency of reflux were also evaluated. The participants with dental erosion had significantly lower values of total proteins, calcium and pH compared to the control group, in both unstimulated and stimulated saliva, indicating an unfavorable biochemical environment for enamel stability. More patients with dental erosion smoke than do patients without dental erosion, and the frequency of regurgitation was different. The combination of decreased calcium and total proteins in saliva indicates a disturbance in the biochemical mechanisms that maintain enamel integrity. The protein depletion presumably contributes to a lowered dielectric strength against erosive challenges, when we consider the established functions of the salivary proteins in the formation of the acquired pellicle, the regulation of mineral ion concentrations, and the protection of surfaces. The data suggest that dental erosion is due to a multi-factorial biochemical imbalance in saliva, involving alterations of acid - base states, decreased availability of minerals, and decreased protective capabilities of proteins, which increases the susceptibility of enamel to erosive destruction.

Keywords: *Sakiva Porteins; Calcium; Saliva pH; Smoking; Dental Erosion.*

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I. INTRODUCTION

Through its natural lubrication, digestion and protection of tissues, saliva plays a vital role in maintaining the equilibrium between the conditions of the mouth [1]. These protective functions seem to depend on salivary flow rate as well as the biochemical properties of the saliva, most notably the salivary proteins [2, 3].

Salivary proteins are also very important to the health of teeth because they help regulate the mineral balance and provide protection to the surface of teeth [2, 3]. Statherin and proline-rich proteins (PRPs) have been shown to stabilize calcium and phosphate ions, thus preventing them from precipitating out of solution, and by doing so, they help to maintain the integrity of enamel [4]. Similarly, proteins that help form the acquired pellicle create a protective layer on the dental surface that reduces the effects of chemical and mechanical challenges to the dental surface [4, 5]. Additionally, some proteins, such as mucins, enhance lubrication, facilitate the aggregation of microbes, and provide surface protection to maintain the balance between demineralization and remineralization processes [6, 7].

The capacity of salivary proteins to provide protect is under the influence of various physicochemical parameters including pH and calcium concentration, both of which influence the structure of the proteins, the ability of the proteins to bind to the teeth and the interactions of the proteins with the teeth [2]. If these parameters are changed, then the functionality of the proteins might also be altered, thus decreasing the efficacy of the salivary mechanisms that are responsible for providing dental defence [8].

Dental erosion is caused by the loss of hard tissue on teeth over time through chemical reactions. Saliva plays a significant role in protecting teeth from damage caused by dental erosion [9]. The buffering of acid in the mouth and the dilution of acids in the mouth, as well as the creation of an acquired pellicle, limit the direct contact between acid and enamel through the use of saliva [10]. The protein composition and buffering capacity of saliva, as well as the presence of calcium, are the main drivers influencing the effectiveness of these protective mechanisms [11].

Changes in the composition of saliva, the functionality of saliva proteins, or the levels of calcium or the level of acidity (pH) in saliva may decrease the effectiveness of the

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protective mechanisms and make teeth more susceptible to dental erosion [9]. Environmental factors, such as having an acidic diet or having a reflux from the stomach, can also affect the composition of saliva and contribute to the development of dental erosion [12,13]. Recent studies have also been published indicating that salivary pH is associated with dental erosion and that the physicochemical properties of saliva play an important role in the erosive processes that can damage teeth [14]. In previous research, smoking has been related to changes in the function of salivary glands, a decrease in salivary volume, and a change in the composition of proteins in saliva. These changes could also lead to a decrease in the protective role of saliva and an increased susceptibility to oral diseases [2].

Therefore, this study aimed to evaluate total salivary protein concentration and its association with salivary pH, calcium concentration, and selected risk factors in individuals with and without dental erosion. By investigating these parameters, the study sought to clarify the potential contribution of salivary proteins to the protective capacity of saliva and their relationship with the presence of erosive lesions.

II. MATERIALS AND METHODS

➢ *Study Design*

The study was designed as a cross-sectional investigation and conducted at the Clinic for Restorative Dentistry and Endodontics, University Dental Clinical Center "Prof. Dr. Bojo Andreski" in Skopje in 2016 [15]. A total of 60 participants aged 18–40 years were enrolled in the study. The study population included individuals of both sexes and participants were divided into two groups, as a study group comprising (n=30) with diagnosed dental erosion and a control group comprising (n=30) without clinical signs of erosive lesions. The study was based on clinical and questionnaire (adapted from the Pickard questionnaire) data originally collected within a master's thesis by Omeragić [15] investigating non-carious lesions. So, the study represents a secondary analysis of these data, focusing specifically on salivary biochemical parameters associated with dental erosion.

➢ *Saliva Collection and Biochemical Analysis*

Unstimulated whole saliva was collected using the spitting method as described by [16]. Participants were instructed to allow saliva to accumulate in the floor of the mouth and expectorate into a graduated test tube every 2 minutes over a 10-minute period. Stimulated whole saliva was collected using the spitting method with mechanical stimulation. Participants chewed paraffin wax pellets for 5 minutes and expectorated saliva into graduated test tubes. Salivary flow rate was calculated as the volume of saliva secreted per minute (mL/min).

The pH of both unstimulated and stimulated saliva samples was measured potentiometrically using a calibrated pH meter (Hanna Instruments, pH Meter 209). The Biuret method is a widely used and reliable analytical approach for protein quantification in biological samples and is consistent with the broader application of spectrophotometric techniques in biochemical analysis [17, 18, 19]. Total calcium concentration in saliva was determined using a photometric method based on the o-cresolphthalein complexone reaction (Human, Germany), under alkaline conditions to form a purple-colored complex, the intensity of which is directly proportional to calcium concentration and was measured spectrophotometrically according to the manufacturer's instructions [20].

➢ *Statistical Analyses*

Statistical analysis was performed using STATISTICA 7.1 and SPSS 17.0. Data were initially processed using descriptive statistical methods, including calculation of mean values and standard deviations. The distribution of variables was assessed using the Shapiro-Wilki`s W test. Depending on the distribution, differences between groups were evaluated using either parametric (Student's t-test) or non-parametric tests (Mann–Whitney U test and Wilcoxon matched pairs test). Categorical data were analyzed using proportion-based comparisons. Statistical significance was defined at a level of $p < 0.05$.

III. RESULTS AND DISCUSSION

The study included 60 participants divided into two equal groups, with and without erosive dental lesions. The comparison between these groups revealed consistent differences in salivary parameters, beginning with pH values, which represent a primary environmental factor influencing enamel stability.

A. pH of Saliva

The analysis pH of saliva revealed consistent differences between the study group, participiants with erosive lesions and the control group. Unstimulated saliva results showed, the mean pH in the study group as 6.2 ± 0.6, compared to 6.5 ± 0.5 in the control group (Table 1, Fig 1).

Table 1 Mean Unstimulated Salivary pH in Both Groups.

pH of unstimulated saliva	Number of participants	Mean	Minimum	Maximum	SD
Experimental group	30	6.2	5.0	7.5	0.636658
Control group	30	6.5	5.5	7.5	0.507518

In the Mann–Whitney U test (Table 2), this difference was statistically significant ($p = 0.039876$), indicating a more acidic baseline environment in individuals with dental erosion.

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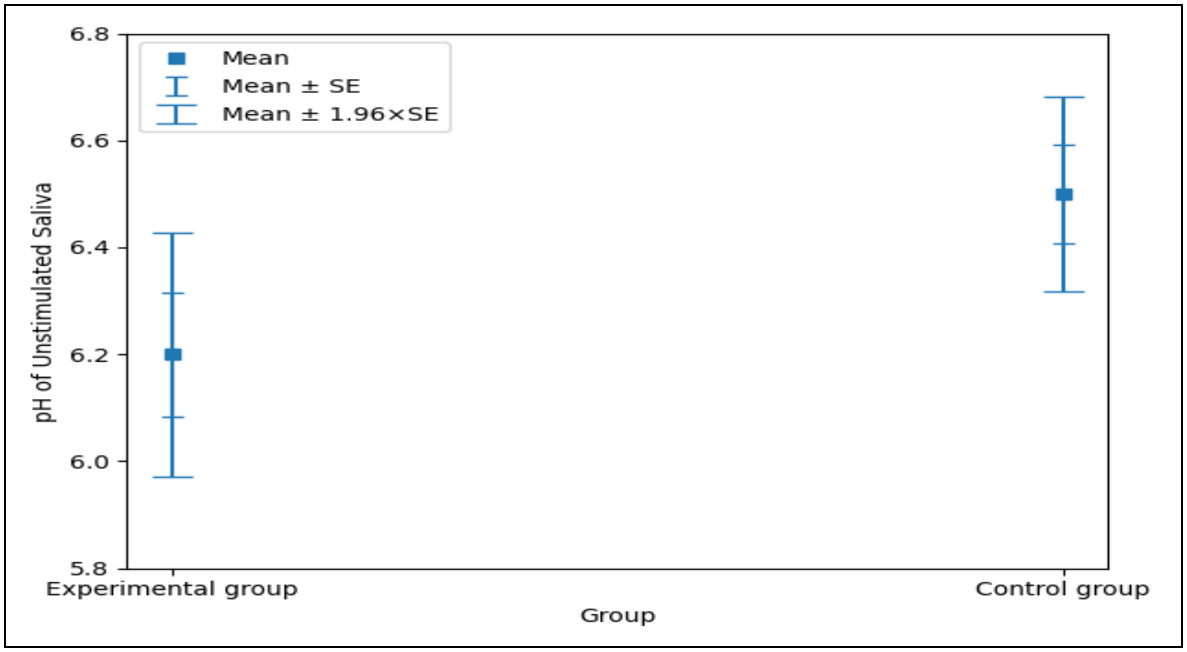

Fig 1 Mean in the Unstimulated Salivary pH.

Table 2 Mann-Whitney U test of Unstimulated Salivary pH

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

In stimulated saliva, the difference between the groups was more pronounced. The study group had a mean pH of 6.7 ± 0.6; the control group had a higher mean of 7.2 ± 0.5 (Table 3 and Fig 2).

Table 3 Mean Stimulated Salivary pH in Both Groups.

pH of stimulated saliva	Number of participants	Mean	Minimum	Maximum	SD
Experimental group	30	6.7	5.5	7.5	0.602116
Control group	30	7.2	6.0	8.0	0.537127

In the Mann–Whitney U test (Table 4), the difference was highly statistically significant (p = 0.001557), suggesting a reduced buffering response under stimulation in the erosion group.

Fig 2 Mean Stimulated Salivary pH in Both Groups

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These results have been confirmed by other researchers in the same subject area, who found that the presence of dental erosion was associated with new changes to salivary factors such as lower pH and a greater incidence of acid exposure in the study population [13,14].

Table 4 Mann-Whitney U Test of Stimulated Salivary pH

pH of stimulated saliva	Rank Sum - Group 1 (Experimental group)	Rank Sum - Group 2 (Control group)	U	Z	p-level
	701.0	1129.0	236.0	-3.16387	0.001557

The pH of saliva is a vital determinant of stability of tooth enamel due to the demineralization process occurring when the pH of saliva drops below the critical point at which hydroxyapatite can dissolve, approximately 5.5 [21]. Moderate and repeated changes in the pH of saliva can significantly shift the balance towards mineral loss, particularly when there is frequent exposure to acidic substances [9]. In addition to supporting this connection, studies demonstrate that dietary acids and intrinsic acids significantly influence the oral-pH dynamics and can contribute to the development of dental erosion [11].

Within-group analysis further demonstrated that stimulation significantly increased salivary pH in both groups (Wilcoxon test, $p < 0.001$) (Table 5). However, despite this increase, the study group maintained lower pH values compared to controls, indicating a persistently more acidic oral environment.

Table 5 Wilcoxon Matched Pairs Test

pH unstimulated/stimulated	Valid - N	T	Z	p-level
Experimental group	30	32,0	4,123952	0,000037
Control group	30	0,00	4,782139	0,000002

Along with having an impact on mineral equilibrium, changes in pH also have a direct effect on the function and structural conformation of salivary proteins and, as a result, may influence the proteins' ability to bind calcium ions and ultimately affect pellicle formation [2]. The function of the proteins is dependent on their environment; therefore, small changes in the pH of saliva may affect their stability, binding affinity, and ability to interact with the enamel surfaces [3,8].

The effect of pH on erosion should in this context be mainly explained through its effect on salivary proteins because changes in protein conformation and binding capacity will have a large impact on how physicochemical changes then reduce the protective function [6, 7].

B. Calcium Concentration in Stimulated Saliva

The calcium concentration in the study group was 1.1 ± 0.3 g/L, whereas in the control group it was 1.6 ± 0.3 g/L (Table 6, Fig 3), and this difference was statistically significant (Mann–Whitney U test, $p < 0.001$), indicating reduced calcium levels in individuals with dental erosion (Table 7).

Table 6 Ccalcium Concentration of Stimulated Saliva in Both Groups.

Concentration of Ca in stimulated saliva (g/L)	Number of participants	Mean	Min.	Max.	SD
Experimental group	30	1.1	0.1	1.6	0.340291
Control group	30	1.6	1.1	2.2	0.277530

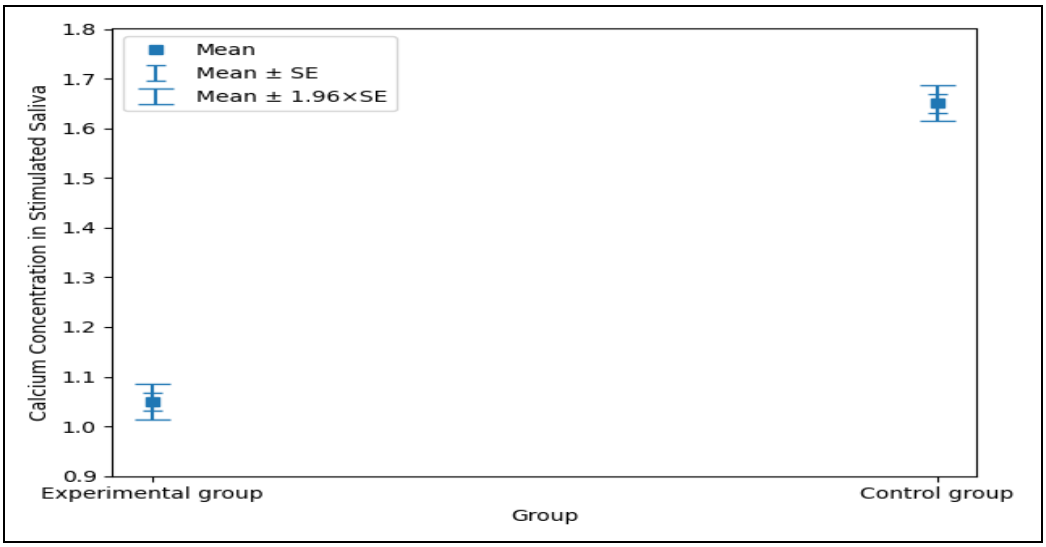

Fig 3 Mean Calcium Concentration of Stimulated Saliva in Both Groups.

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Teeth, as well as other tissues, are made up of a group of minerals called hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). During natural processes within our mouths, we frequently add and take away minerals from our teeth. The major mineral source is saliva, and the quantity and composition of calcium ions in saliva will assist with maintaining the balance of the minerals within our teeth. Saliva contains two types of calcium: free (ionized) and protein-bound; in both forms of calcium, the balance between the two is maintained by proteins found in saliva. Salivary calcium-binding proteins, such as statherin and proline-rich proteins, work together to stabilize (or prevent precipitation) of calcium ions and maintain a level of excess mineral content relative to hydroxyapatite [5].

Table 7 Mann-Whitney U test – Ca Concentrations of Stimulated Saliva.

Concentration of Ca in stimulated saliva	Rank Sum (Experimental group)	Rank Sum (Control group)	U	Z	p-level
	535.0	1295.0	70.0	-5.6180	0.0000

The functional bioavailability of the free and protein-bound calcium in saliva is largely dependent on the protein-calcium interaction within saliva; the calcium in saliva that is bound to proteins is a stable fraction in saliva that participates directly in the process of acquired pellicle formation and mineral stabilization [3, 4]. From the perspective of chemical supersaturation of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), saliva is almost always in a state of supersaturation relative to hydroxyapatite, which will prevent the demineralization of enamel and facilitate the remineralization of enamel. Supersaturation occurs in saliva due to the presence of calcium (Ca^{2+}), phosphate (PO_4^{3-}), and hydroxyl (OH^-) ions. Upon encountering an acid challenge, H^+ ions will displace OH^- and PO_4^{3-} and shift the equilibrium state toward demineralization [1, 8, 9]:

$$Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \rightleftharpoons 10Ca^{2+} + 6HPO_4^{2-} + 2H_2O$$

The decrease in pH of saliva below a specific critical threshold (approximately pH 5.5) will prevent the accumulation of free calcium ions or free phosphorus ions to maintain calcium/phosphate supersaturation; therefore, there will be a net loss of minerals from the tooth.

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C. Salivary Proteins in Stimulated Saliva

Analyzing total salivary protein content within stimulated saliva, there are substantial differences seen when comparing the study group (subjects with erosive lesions) to those within the control group.

The mean (± SD) salivary protein concentrations for each group were 1.3 ± 0.4 g/L for the study group versus 2.3 ± 0.9 g/L for the control group (Table 8, Fig 4). This difference is statistically significant (Mann–Whitney U test, $p < 0.001$) and indicates there is a reduced total salivary protein content in individuals who have dental erosion (Table 9).

Table 8 Mean Concentration of Salivary Proteins in Stimulated Saliva.

Salivary proteins of stimulated saliva (g/L)	Number of participants	Mean	Minimum	Maximum	SD
Experimental group	30	1.3	0.4	2.0	0.422870
Control group	30	2.3	0.1	3.8	0.889278

The total salivary proteins are a wide-ranging collection of proteins that have a variety of functions. Some of these functions include: catalyzing enzymatic reactions (amylase), forming mucin (antimicrobial) compounds to inhibit microbial growth, and acting as calcium-binding proteins (statherin, proline-rich proteins). In saliva that has been stimulated, especially from the parotid gland, α-amylase contributes a large percentage to the total amount of protein found in that saliva (approximately 40-50%). The proline-rich proteins contribute about 20-30% and, while statherin only contributes a small amount to the overall % of protein found in saliva (1-3%), it is an important contributor to the function of saliva [5]. These proteins, despite their varying amounts, work together to create and maintain a constant oral environment.

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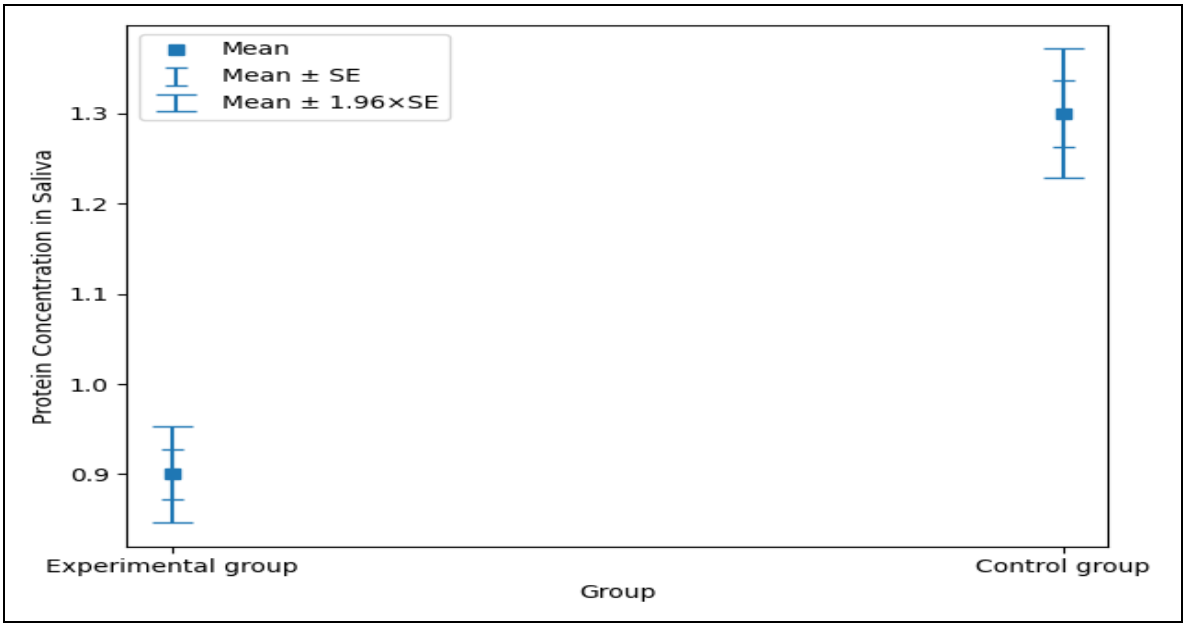

Fig 4 Mean Salivary Protein Concentration in Stimulated Saliva in Both Groups

The primary functional role of salivary proteins is to maintain mineral equilibrium. The calcium-binding proteins (statherin, proline-rich proteins) stabilize calcium and phosphate ions, prevent the spontaneous precipitate formation, and maintain supersaturation with hydroxyapatite in order to maintain enamel structure [4]. In addition, salivary proteins are also necessary for forming the acquired pellicle, a proteinaceous barrier that protects the enamel from direct acid contact. Within this functional framework, salivary proteins can be considered the central mediators of oral protection, as both mineral regulation and barrier formation are directly dependent on their structural integrity and binding capacity [3, 4].

Table 9 Mann–Whitney U test of salivary protein concentration in stimulated saliva.

Salivary protein concentration in stimulated saliva	**Rank Sum (Experimental group)**	**Rank Sum-Group 2 (Control group)**	**U**	Z	**p-level**
	604.0	1226.0	139.0	-4.59796	< 0.001

Based on reductions in total salivary proteins measured in individuals with acidic exposure, salivary protection appears to have decreased due to a decrease in the protein concentration in saliva and provides an additional layer of meaning to the relationship between acidic exposure and reduced salivary protective capacity. The mechanisms underlying decreased salivary proteins include the possibility that salivary proteins will decrease due to reduced secretion from the salivary gland(s), changes in salivary gland function, or alterations in stability of salivary proteins, all of which may diminish the ability of calcium to bind to proteins in the salivary pellicle and protect against acidic attacks [22]. During stimulated salivary flow, while an increase in flow will generally decrease the concentration of proteins in saliva, the protein concentration under stimulation does provide evidence of coordinated secretion from the glands, while reductions in major proteins (i.e., α-amylase) indicate overall reductions in other large groups of salivary proteins [2]. Further reduction in protein levels due to exposure to acidic environments could be from acidic exposure adversely affecting saliva production and/or secretion [2], along with decreasing the pH of saliva protein and thus causing changes in conformation of proteins to further negatively impact interaction between saliva proteins, calcium, and enamel.

The secondary method appears to be supported by both the current study and from previous work done, in which increased time spent in an acidic environment has been shown to decrease pH of saliva [13, 14], suggesting that salivary protein stability and functionality are modulated by environmental conditions through changes in the physicochemical environment.

In summary, the two mechanisms are likely interdependent and demonstrate that protein functionality may become inhibited by exposure to acids while protein levels decrease from exposure to acidic environments. The interaction between salivary proteins and mineral ions can be represented as:

$Ca^{2+} + PO_4^{3-} + \text{proteins} \rightleftharpoons \text{protein–mineral complexes}$

The calcium and phosphate binding properties of salivary proteins contribute to maintaining mineral homeostasis of the tooth structure and protecting the tooth enamel [2, 4, 7]. Enamel proteins found in mature enamel affect not only the mechanical properties of enamel, but also contribute to resisting acid attacks. The natural presence of proteins in the enamel provides a passive means of protection that slows down acid from reaching the surface of enamel

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crystallites and hence, slows the progression of erosive wear [23, 24].

The findings from this study show that the significantly lower concentration of proteins in the study group demonstrates that salivary proteins are instrumental in maintaining a protective capacity throughout the oral cavity.

Table 10 Gastric reflux and t-test in weekly and monthly frequency.

Regurgitation	Mean (Experimental group)	Mean (Control group)	t-test	df	p	N (Experimental group)	N (Control group)	SD (Experimental group)	SD (Control group)
Weekly	12.0	1.6	1.3	30	0.190238	29	30	13.15566	1.154701
Monthly	23.2	3.0	4.5	40	0.000052	29	30	15.26863	2.088932

Further, the changes in protein quantity and functionality are related to changes in erosion and are best understood within a system that includes pH, calcium, and environmental factors [25,26].

D. Smoking and Gastric Reflux Effect

The analysis showed a higher effect of smoking in the experimental group (76.7%) compared to the control group (26.7%) (Fig 5), were smoking is known to affect salivary gland function and reduce protective proteins, there by altering oral defense mechanisms [2, 24].

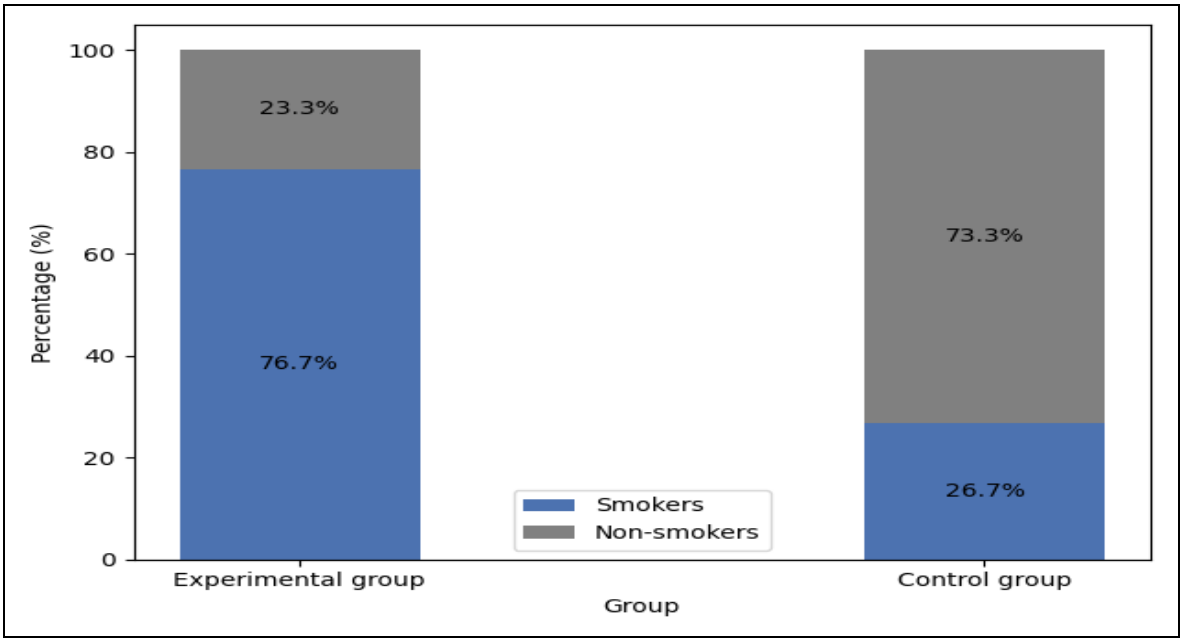

Fig 5 Smoking habits of participants.

Gastric reflux frequency was significantly higher in the experimental group, particularly for monthly episodes, indicating greater exposure to intrinsic acids. Monthly condition (Table 10) shows a clear, statistically significant difference between groups ($t = 4.5$, $p < .001$). The experimental group averaged 23.2 vs. the control's 3.0 — a roughly 7-fold difference. Weekly condition did not reach significance ($t = 1.3$, $p = .190$), despite the experimental group mean (12.0) being much higher than control (1.6). The likely culprit is the very large SD in the experimental group (13.16) relative to its mean — high within-group variability reduces statistical power (Table 10).

Salivary protein structure may have been compromised by both acid exposure and as a result of a lower pH of saliva [13, 14], which disrupts intramolecular interactions; therefore, when salivary proteins are exposed to acid the potential to form a pellicle will be adversely affected [9]. Reflux and smoking seem to have modified risk factor effects and change the salivary environment, probably through changes in salivary proteins. Salivary proteins are critical to calcium stabilization, mineral balance, and enamel protection. The observed lower levels of total protein and calcium further support saliva in the experimental group [27] will have less protection through the salivary properties.

IV. CONCLUSION

The data collected throughout the course of this research demonstrates that dental erosion can alter the composition of saliva in a way that reduces its effectiveness as a protective fluid. These alterations result in lower levels of calcium, lower levels of total salivary proteins, as well as lower pH values. All three of these variables will negatively impact the ability of saliva to protect against dental erosion through a mechanism that consists of a reduction of buffering capacity, reduced ability for remineralization and the ability to form an acquired pellicle on the enamel surface. Salivary

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proteins are essential to the maintenance of oral health because they bind calcium, alter the mineral balance of saliva, lubricate, and protect the enamel surface from acidic damage. Therefore, when total saliva protein is reduced, along with a decrease in calcium levels, it is likely that the synergistic effects of these two alterations will decrease the ability of saliva to protect enamel from the effects of demineralization. In particular, the data that indicates a significant decrease in salivary protein in the study population demonstrates the importance of salivary proteins in the overall capacity of saliva to protect against dental erosion. This study provides evidence supporting the theory that changes in the protein composition and concentration of saliva are key biochemical determinants in the development and progression of dental erosion and act as very useful markers for an individual's risk of developing dental erosion. Future studies using specific salivary proteins and their relationship with the mineral constituents of saliva, in order to better define the role of salivary proteins in the prevention and progression of erosive tooth structure loss, should be pursued.

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