IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 22, Issue 7 Ser.2 (July. 2023), PP 19-26
www.iosrjournals.org

Estimation Of Salivary Alpha Amylase Levels And Salivary Flow Rate And Their Correlation With Periodontal Status: A Clinico-Biochemical Study

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Abstract
Background: *Saliva Is A Body Fluid Containing A Complex Mixture Of Products Secreted Mainly By The Salivary Glands. Inorganic And Organic Secretory Products May Play Different Biological Roles In Digestion, Host Defense And Lubrication. The Protein Composition Of Saliva And The Flow Rate May Be Affected By Periodontal Disease. This Study Aims To Estimation And Compare Salivary Alpha Amylase Levels And Salivary Flow Rate In Healthy, Gingivitis And Chronic Periodontitis Subjects.*
Materials And Method: *A Total Of 30 Subjects (25-55 Years, 13 Males And 17 Females) Were Selected From The Department Of Periodontics, Goa Dental College And Hospital, Bambolim, Goa And Were Assigned Into 3 Groups Consisting Of 10 Subjects Each: Group A (Healthy Control), Group B (Gingivitis), Group C (Chronic Periodontitis). All The Subjects Underwent A Full Mouth Periodontal Examination Which Included The Following Periodontal Parameters Such As Bleeding On Probing (Bop), Gingival Index (Gi), Plaque Index (Pi), Pocket Probing Depth (Ppd) And Clinical Attachment Level (Cal) Recorded Using A University Of North Carolina (Unc)15 Probe. Unstimulated Saliva Samples (1 Ml) Were Collected By Allowing Saliva To Flow Passively Into A Sterile Tube (Without Stimulation) For The Estimation Of Salivary Amylase Levels And Salivary Flow Rate.*
Results: *No Statistically Significant Difference Was Observed Between The Age And Gender Among The Three Groups. The Difference Between The Mean Values Of Salivary Alpha Amylase (P=0.498) And Salivary Flow Rate (P=0.750) Among The Three Study Groups Was Statistically Non-Significant. Highly Statistically Significant Difference Was Observed In Bop In Group B (P=<0.001). Statistically Significant Difference Was Observed In Pi In Group A And Group B (P=<0.012, P=0.022 Respectively). Statistically Significant Difference Was Observed In Gi In Group A (P=0.042). No Statistically Significant Difference Was Observed In Ppd And Cal Among The Three Groups (P>0.05). Pair Wise Group Comparison Of Salivary Alpha Amylase And Salivary Flow Rate Showed Statistically Non-Significant Difference. Pearson's Correlation Test Revealed A Negative Correlation Between Salivary Alpha Amylase, Salivary Flow Rate And Clinical Periodontal Parameters (P>0.05).*
Conclusion: *Salivary Alpha Amylase Levels And Salivary Flow Rate Did Not Show Any Correlation With Periodontal Status Of The Patient. However, Longitudinal Clinical Trials With A Larger Sample Size May Be Necessary For Better Understanding Of This Correlation In The Near Future.*
Key Words*: Alpha Amylase, Flow Rate, Gingivitis, Chronic Periodontitis*

Date of Submission: 24-06-2023 Date of Acceptance: 04-07-2023

I. INTRODUCTION

Periodontal disease is a complex infectious condition resulting from an interplay of microbial infection and host-immune response to the microbial challenge, which is modified by certain environmental and acquired risk factors, and genetic susceptibility of the host.[1] Diagnosis of periodontal disease is usually based on traditional diagnostic methods which involves clinical parameters, such as pocket probing depth (PPD), bleeding on probing (BOP), clinical attachment level (CAL) and radiographic methods, which have been widely used and well documented. However, these traditional diagnostic methods fail to provide notable information on current disease activity.[2]

Over the years, saliva has been successfully used as a diagnostic tool with the help of biomarkers. A biomarker is a substance which is measured objectively and evaluated as an indicator of normal biologic or pathologic process, or pharmacologic response to a therapeutic intervention.[3] Research studies have explored the use of different salivary biomarkers in the diagnosis and monitoring of oral diseases. Composition of saliva includes numerous enzymes including alpha amylase, whose potential as a biomarker has not been clearly understood.[4, 5]

Salivary alpha amylase is a calcium containing metalloenzyme which is produced mainly by the parotid gland. Its functions include break down of high molecular weight carbohydrates to lower molecular weight sugars (i.e., glucose), maintaining the mucosal immunity and preventing adherence of streptococcal species, which may inhibit further propagation of bacterial colonization and aid in regulating normal microbial flora in the mouth. It also possesses anti-microbial properties and thus may participate in non-immunological defense mechanism of the oral cavity against various periodontal pathogens.[6,7]

It has been suggested that periodontal health may be linked to salivary function owing to its mechanical cleansing action and anti-microbial property.[8] However, insufficient data is available to support this association between salivary flow rate and periodontal health. Therefore, the present study was carried out with the aim to estimate and compare the levels of salivary alpha amylase and salivary flow rate in healthy patients, patients with gingivitis and chronic periodontitis.

II. MATERIALS AND METHOD

A total of 30 systemically healthy subjects (13 males and 17 females), aged between 25 and 55 years were selected from the outpatient Department of Periodontics, Goa Dental College and Hospital, Bambolim, Goa. The study protocol was approved by the Institutional Ethical Committee of Goa Dental College and Hospital, Bambolim. Pregnant and lactating women, smokers, chronic alcoholics, patients with history of use of antibiotics within past six months and those patients who received periodontal treatment within past six months were excluded from the study. All the patients were well informed about the purpose of the study and those patients willing to participate in this study signed the written informed consent.

Patients were then assigned into 3 groups consisting of 10 subjects each.

Group A: Healthy group (control group): No evidence of clinical inflammation, sulcular bleeding or clinical attachment loss.

Group B: Gingivitis group: Presence of BOP, clinical inflammation but no evidence of clinical attachment loss.

Group C: Chronic periodontitis group: >30% of sites involved, moderate to severe alveolar bone loss, clinical attachment loss > 3mm and PPD ≥5mm and the amount of destruction consistent with local factors.

All the subjects underwent a thorough case history-taking and clinical examination. After appropriate grouping of the subjects, a full mouth periodontal examination was performed by a single examiner which included periodontal parameters such as bleeding on probing (BOP), gingival index (Loe and Silness, 1963), plaque index (Silness and Loe, 1964), pocket probing depth (PPD) and clinical attachment level (CAL) recorded using a University of North Carolina (UNC)15 probe.

BIOCHEMICAL ANALYSIS

Collection of saliva for estimation of alpha amylase[9]

Collection of saliva sample was performed in the morning between 9.00-11.00 a.m. with study patients sitting comfortably in an upright position. The patients were informed to refrain from exercising, eating, smoking, drinking any beverages except water 1 hour prior to saliva sampling. After rinsing the mouth with water to wash out exfoliated cells, patients were instructed to wait for 5 minutes and to spit out or swallow the saliva that is already present in the mouth before collection of the sample. Unstimulated saliva samples (1 ml) were collected by allowing saliva to flow passively into a sterile tube (without stimulation). Analysis of sample was performed immediately after collection of the saliva. Saliva samples were centrifuged at 3000 rpm for 20 min. The upper part was drawn and used for estimation of salivary alpha amylase.

Estimation of salivary alpha amylase[9]

Serum alpha amylase (SAA) level was measured using a kinetic assay method using a commercially available α-AMYLASE KIT (Coral Clinical Systems, A division of Tulip Diagnostics (P) Ltd, Verna, Goa, India).

Principle:

α- Amylase catalyses the hydrolysis of a 2- chloro- 4 nitrophenol salt to chloro-nitrophenol (CNP). The rate of hydrolysis is measured as an increase in the absorbance due to the formation of chloro-nitrophenol which is proportional to the α- Amylase activity in the sample.

α- Amylase

CNP – Gal – G2 + H_20 ⟶ CNP + Gal -G2

To perform the α- Amylase test, 1000 µL of amylase monoreagent was taken into a sterile glass tube using a calibrated micropipette. 20 µL of salivary supernatant was pipetted into the same glass test tube and mixed well. This reaction resulted in yellow colour due to the presence of α- amylase. This was followed by waiting for 60 seconds, to assess SAA level with an autoanalyzer.

Measurement of salivary flow rate

Unstimulated salivary flow rate was measured using the draining method.[10] To measure the unstimulated salivary flow rate, the patients were asked to sit straight and bend their head slightly forward. The patients were first asked to swallow, and then to drool saliva for 5 minutes into a saliva collection tube.

$$\text{Salivary flow rate (gm/ml)} = \frac{\text{Post weight measure} - \text{Pre weight measure}}{\text{Collection period}}$$

[* Salivary gland hypofunction is considered when whole unstimulated saliva is < 0.1 g/minute (that is, mL/minute), whole chewing stimulated saliva is < 0.7 g/minute or both.]

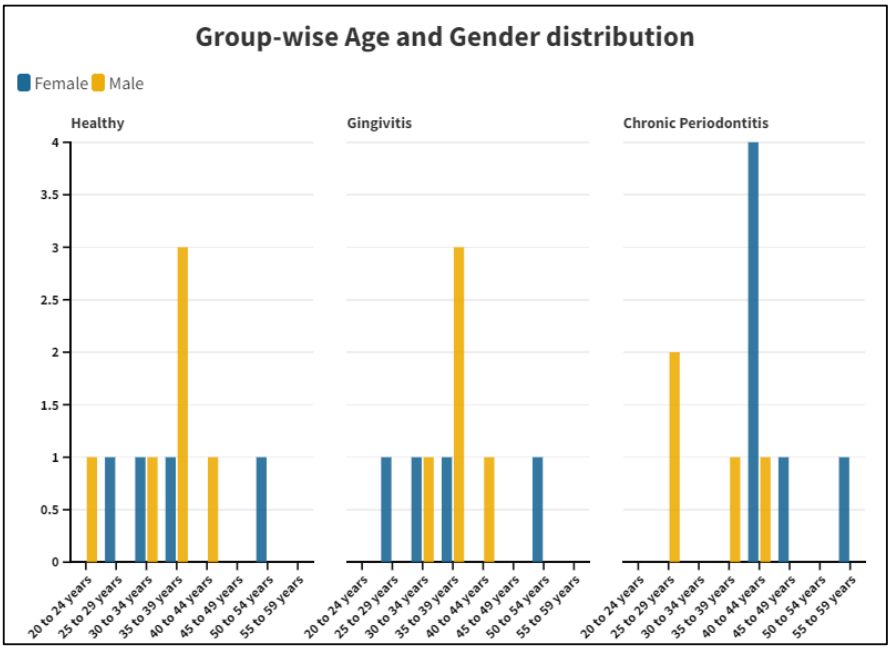
STATISTICAL ANALYSIS

The data was analyzed using a STRATA software version 17.0. Comparison of groups A, B and C at baseline was done using ANOVA test.Intragroup comparison for group B and C with group A was done at baseline. p <0.05 was considered statistically significant. Data was checked by Shapiro Wilk test. Level of significance was set at 5%.

III. RESULTS

The mean age of subjects in Group A, Group B and Group C was 35.80±8.40, 36.90±5.02 and 40.60±8.88 respectively. The difference between the mean age among the three study groups was statistically non-significant (p= 0.447) (Graph no.1).

Genderwise, Group A consisted of 60% males (n=6) and 40% females (n=4) with a mean of 1.40±0.52. Group B consisted of 30% males (n=3) and 70% females (n=7) with a mean of 1.70±0.48 . Group C consisted of 40% males (n=4) and 60% females (n=6) with a mean of 1.60±0.52. No statistically significant difference was found between the gender among the three groups (p=0.429) (Graph no.1).



Graph No. 1: Group-wise Age and Gender distribution

Salivary alpha amylase

The mean value of salivary alpha amylase in Group A, Group B and Group C was 68.50±19.86, 58.29±36.87 and 55.58±30.46 respectively. The difference between the mean values of salivary alpha amylase among the three study groups was statistically non-significant (p=0.498) (Table no. 1).

Table no. 1: Salivary alpha amylase values across the groups

Group	Mean	Standard Deviation	Shapiro-Wilk W	Shapiro Wilk p	One-way ANOVA	
					F	P value
Healthy	68.50	19.86	0.94	0.510	0.73	0.498
Gingivitis	58.29	36.87	0.83	0.030		
Chronic Periodontitis	55.58	30.46	0.87	0.097		

Salivary flow rate

The mean value of salivary flow rate in Group A, Group B and Group C was 0.26±0.17, 0.21±0.14 and 0.22±0.11 respectively. The difference between the mean values of salivary alpha amylase among the three study groups was statistically non-significant (p=0.750) (Table no. 2)

Table no. 2: Salivary flow rate values across the groups

Group	Mean	Standard Deviation	Shapiro-Wilk W	Shapiro Wilk p	One-way ANOVA	
					F	P value
Healthy	0.26	0.17	0.92	0.335	0.29	0.750
Gingivitis	0.21	0.14	0.89	0.160		
Chronic Periodontitis	0.22	0.11	0.80	0.013		

Pair wise Group comparison using Tukey's Post Hoc Test (Table no. 3)

Pair-wise group comparison of salivary alpha amylase showed that the mean difference between Group A & B was -10.21 (p=0.728), Group A & C was -12.92 (p=0.604) and Group B & C was -2.71 (p=0.978). The difference was found to be statistically non-significant.

Pair-wise group comparison of salivary flow rate showed that the mean difference between Group A & B was -0.05 (p=0.697), Group A & C was -0.04 (p=0.791) and Group B & C was 0.01(p=0.986). The difference was found to be statistically non-significant.

Dependent Variable	**(I) Group**	**(J) Group**	**Mean Difference (I-J)**	**p value**
Salivary Alpha Amylase	Healthy	Gingivitis	-10.21	0.728
	Healthy	Chronic Periodontitis	-12.92	0.604
	Gingivitis	Chronic Periodontitis	-2.71	0.978
Salivary Flow Rate	Healthy	Gingivitis	-0.05	0.697
	Healthy	Chronic Periodontitis	-0.04	0.791
	Gingivitis	Chronic Periodontitis	0.01	0.986

Table no. 3: Pair -wise Group comparison using Tukey's Post Hoc Test

Mean values of clinical parameters (Table no. 4, Graph no.2):

The mean values of BOP for subjects in Group A, Group B and Group C was 0, 0.80±0.42 & 1.00 respectively. Highly statistically significant difference was found in Group B (p=<0.001).

The mean values of PI for subjects in Group A, Group B and Group C was 1.24±0.12, 1.52±0.15 and 1.97±0.21 respectively. Statistically significant difference was found in Group A and Group B (p=<0.012, p=0.022 respectively).

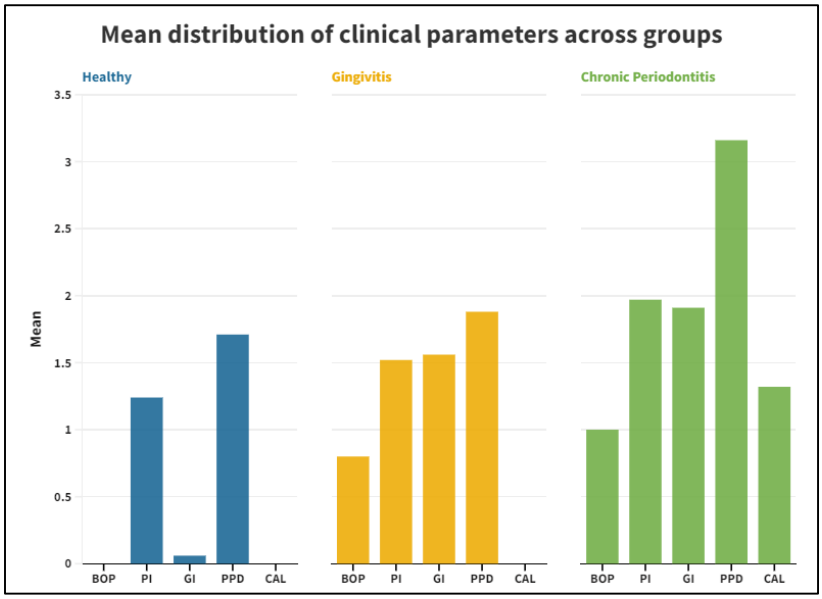
The mean values of GI for subjects in Group A, Group B and Group C was 0.06±0.03, 1.56±0.15 and 1.91±0.16 respectively. Statistically significant difference was found in Group A (p=0.042).

The mean values of PPD for subjects in Group A, Group B and Group C was 1.71±0.16, 1.88±0.13 and 3.16±0.21 respectively. No statistically significant difference was found among the three groups (p>0.05).

The mean values of CAL for subjects in Group A, Group B and Group C was 0.00, 0.00 and 1.32±0.19 21 respectively. No statistically significant difference was found among the three groups (p>0.05).

Clinical Parameters	Healthy				Gingivitis				Chronic Periodontitis			
	Mean	Standard Deviation	Shapiro Wilk W	Shapiro Wilk p	Mean	Standard Deviation	Shapiro Wilk W	Shapiro Wilk p	Mean	Standard Deviation	Shapiro Wilk W	Shapiro Wilk p
BOP	0	0	NaN	<NaN	0.80	0.42	0.51	**<0.001***	1.00	0.00	NaN	NaN
PI	1.24	0.12	0.79	**<0.012***	1.52	0.15	0.81	**0.022***	1.97	0.21	0.91	**0.310**
GI	0.06	0.03	0.84	**0.042***	1.56	0.15	0.97	**0.886**	1.91	0.16	0.91	**0.295**
PPD	1.71	0.16	0.89	**0.182**	1.88	0.13	0.94	**0.575**	3.16	0.21	0.97	**0.868**
CAL	0.00	0.00	NaN	NaN	0.00	0.00	NaN	NaN	1.32	0.19	0.87	**0.094**

Table no. 4: Mean values of clinical parameters across the groups



Graph no. 2: Mean distribution of clinical parameters across the groups

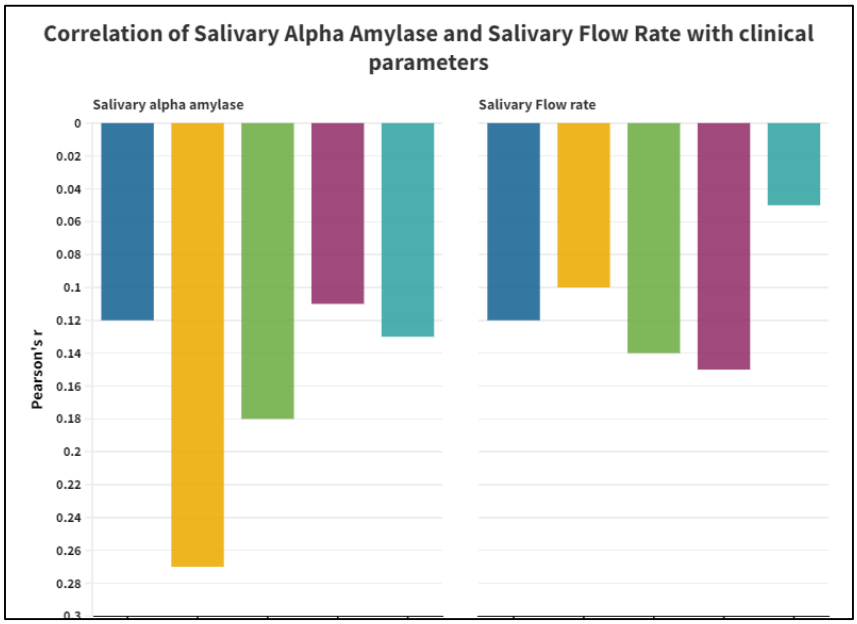
Correlation of Salivary Alpha Amylase and Salivary Flow Rate with clinical parameters (Table no. 5, Graph no. 3):

Pearson's correlation test revealed a negative correlation between salivary alpha amylase and clinical periodontal parameters i. e., r= -0.12, -0.27, -0.18, -0.11 and -0.13 w.r.t. BOP, PI, GI, PPD and CAL respectively. However, the difference was statistically non-significant (p>0.05).

Pearson's correlation test between salivary flow rate and clinical periodontal parameters revealed a negative correlation i.e., r= -0.12, -0.10, -0.14, -0.15 and -0.05 w.r.t. BOP, PI, GI, PPD and CAL respectively. However, the difference was statistically non-significant (p>0.05).

Clinical Parameters	Salivary alpha amylase		Salivary Flow rate	
	Pearson's r	P value	Pearson's r	P value
BOP	-0.12	0.514	-0.12	0.520
PI	-0.27	0.157	-0.10	0.605
GI	-0.18	0.353	-0.14	0.445
PPD	-0.11	0.545	-0.15	0.443
CAL	-0.13	0.477	-0.05	0.794

Table no. 5: Correlation of salivary alpha amylase and flow rate with clinical parameters



Graph no. 3: Correlation of salivary alpha amylase and flow rate with clinical parameters

IV. DISCUSSION

The diagnosis of active phases of periodontal disease, and the identification of patients at risk, constitutes a challenge for the clinicians. As traditional diagnostic methods such as clinical and radiographical evaluation only provide information about the disease severity, there is a need for a simple and rapid diagnostic test that can provide reliable information of periodontal disease activity and identify patients at risk for development of periodontal disease.[11,12]

Saliva is an oral fluid that contains locally and systemically derived markers of periodontal disease, which may offer the basis for a patient-specific biomarker analysis for periodontal disease.[13] Also, the systemic effects of chronic periodontitis induced by transient bacteraemia and the presence of the elevated levels of pro-inflammatory cytokines may affect salivary gland function which may further result in a decreased salivary output.[14]

According to the findings of the present study, PI and GI in healthy subjects ($p<0.012$, $p=0.042$ respectively) and BOP and PI in gingivitis subjects ($p<0.001$, $p=0.022$ respectively) showed statistically significant difference. No significant association was observed between levels of salivary alpha amylase and salivary flow rate ($p=0.498$, $p=0.750$) respectively among the three groups. Correlation of salivary alpha amylase and salivary flow rate with clinical periodontal parameters revealed a negative correlation. However, the results were statistically non-significant ($p>0.05$).

In a study by Hernandez-Castaneda AA et al[15], increased salivary amylase concentration was observed in patients with periodontal disease, but the results showed statistically non-significant difference ($p>0.05$). Similarly, studies by Haririan H et al[17] and Acquier AB et al[17] demonstrated non-significant differences in SAA levels between healthy, chronic periodontitis and aggressive periodontitis patients.

Findings of the present study are in contrast with previously performed studies (Goncalves Lda R et al[18], Sanchez GA et al[19], Kejriwal S et al[6] and Bharadwaj P et al[20]) that showed significantly higher values of salivary alpha amylase in gingivitis and periodontitis groups. These results of increased SAA levels in periodontal diseases were supported on the basis of following reasons[9]:

i) response of salivary glands to inflammatory diseases like gingivitis and periodontitis resulting in increased production and secretion of SAA to augment the defense mechanism of the oral cavity,

ii) alpha amylase may act as a major lipopolysaccharide binding protein of periodontal pathogens such as Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis which may be involved in interference with bacterial adherence and biofilm formation,

iii) increased levels could partially be attributed to an increased leakage of plasma proteins into saliva due to inflammation.

Studies performed by Thakur NP et al[9] and Sanchez GA et al[7] evaluated SAA levels in healthy and chronic periodontitis subjects before and after scaling and root planing which demonstrated reduction of SAA levels following scaling and root planing. Periodontal treatment such as scaling and root planing involves removal of the plaque biofilm and calculus, is responsible for reduction in the bacterial load on periodontium, which in turn lowers the immune response by the host, leading to a decrease in SAA levels.[9]

The relationship between salivary flow and periodontal health was assessed in the present study wherein it was observed that the mean difference for flow rate between the groups was statistically non-significant. This was in agreement with the study by Hirotomi T et al[21], in which the relationship between flow rate and the progression of periodontal disease in elderly population was found to be non-significant.

Studies by Vallabhan CG et al[14] and Sanchez et al[19] have demonstrated a decrease in the salivary flow rate with increasing severity of periodontitis. These findings were attributed to the inflammatory changes in periodontitis that might exert deleterious effects on secretory and functional capacity of salivary gland. The presumed mechanisms might be due to increased production of pro-inflammatory cytokines such as interleukin IL-2, IL-17, and tumor necrosis factor-β (TNF-β) in periodontitis which may trigger glandular dysfunction and resultant hyposalivation. Moreover, the increased accumulation of reactive oxygen species (ROS) in periodontitis may be responsible for causing structural alterations in salivary gland tissue.[14]

Another study by Sinor & Azirrawani[22] and Amalina R et al[23] found a significant increase in salivary flow rate in patients with periodontal disease which contradicted the results of aforementioned studies. These results were explained by the fact that the increased inflammatory activity occurring in periodontal disease may trigger the salivary innervations to increase the salivary flow rate or it could be due to the protective effect of saliva itself, towards the inflammatory process.

In the present study, estimation of salivary alpha amylase levels was performed to evaluate the role of this enzyme in periodontal disease so as to explore its use as a potential biomarker of periodontal disease activity. Additionally, salivary flow rate was also measured to assess the effect of periodontal disease on the secretory function of the salivary gland. The results of the present study demonstrated statistically non-significant difference between salivary alpha amylase levels and salivary flow rate in patients with periodontal disease ($p>0.05$). A negative correlation was observed between salivary alpha amylase and salivary flow rate with the clinical periodontal parameters. However, the difference was statistically non- significant ($p>0.05$).

The limitations of the present study included limited sample size, no interventional treatment was performed, no microbiological analysis was performed, and no additional enzymes or biomarkers were assessed.

V. CONCLUSION

Within the limitations of the study, salivary alpha amylase levels and salivary flow rate did not show any correlation with periodontal status. However, longitudinal clinical trials with a larger sample size may be necessary for better understanding of this correlation in the near future and to further evaluate the role salivary alpha amylase potential biomarker for periodontal disease.

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