



## Evaluation of salivary alpha amylase as an inflammatory biomarker in chronic periodontal disease: a cross-sectional study

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### Abstract

**Introduction:** In systemic and oral diseases, salivary alpha amylase is used as a biomarker. Salivary amylase is elevated in these diseases to play a protective role during the inflammatory stage because all systemic and periodontal diseases are chronic inflammatory diseases.

**Objective:** To evaluate the salivary amylase concentration as inflammatory biomarker in chronic periodontal disease patients.

**Methodology:** 160 subjects were allocated into groups I, II, III, and IV (40 healthy controls, 40 generalized chronic gingivitis, 40 localized and 40 generalized chronic periodontitis). Saliva that had not been stimulated was collected, and amylase was estimated using the kinetic assay method. Independent t-tests, post hoc Tukey, and ANOVA were used to analyze the results.

**Results:** In this study, generalized chronic gingivitis, localized chronic periodontitis, and generalized chronic periodontitis all had significantly higher salivary amylase levels than healthy controls ( $p < 0.001$ ). Salivary amylase level was a bit more in women as compared to men.

**Conclusion:** It is concluded that patients with chronic periodontal disease had higher concentrations of salivary alpha amylase than did periodontally healthy controls. Salivary alpha amylase level concentration was slightly higher in females than male subjects.

**Keywords:** Biomarkers; amylase; periodontal disease; gingivitis; periodontitis.

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## **Introduction**

Periodontal diseases are brought on by the interaction of a pathogenic bacterial biofilm in the periodontal pocket with inflammatory cells and molecules derived from the host. It leads to loss of tooth in adults and brought on by plaque biofilm, which causes gingivitis. Periodontitis, which leads to the loss of bone support and connective tissue, can develop from gingivitis.<sup>1</sup> Conventional techniques for determining periodontal disease rely on clinical parameter evaluation, which is historically constrained and makes it impossible to determine the current disease state or the likelihood of future disease. To address this shortcoming of traditional methods, the field of biomarkers has entered a new era. An objectively measured and assessed substance is referred to as a biomarker if it can be used to predict a pathogenic process, a normal biologic process, or the pharmacologic effects of a therapeutic intervention.<sup>2</sup> Biomarkers for periodontal disease and health are identified using a variety of biological media, including saliva, serum, and GCF. One of the key proteins that makes up 60% of all the proteins the salivary gland produces is the enzyme salivary -amylase (SAA).<sup>3</sup> Locally, salivary glands in the oral cavity produce salivary alpha amylase through the process of exocytosis in response to adrenergic stimulation.<sup>4</sup> Since saliva is a vital part of the host's oral immune defense, patients with periodontal disease have different protein compositions.<sup>5</sup> Study of saliva is favorable as it is a non-invasive method in the determination of periodontal disease.<sup>6</sup> A very limited data is available about salivary alpha amylase level which have been evaluated as biomarker among patients with periodontal disease. Therefore, this research was aimed to assess salivary alpha amylase level concentration as inflammatory biomarker in chronic periodontal disease patients.

## **Materials & Method**

This cross-sectional study was conducted in the Dept. of Periodontology, RDCH&RC Kanpur, Uttar Pradesh. The ethical clearance was taken from Institutional Ethics Committee of RDCH&RC Kanpur, U.P. (Approval number RDCHRC/ETHICSCOMMITTE/2020-21/059) and the motive of the research was described to every participant, informed written consent taken from all study subjects. Every study participant's biodata and case history were recorded in the case history proforma. Saliva samples were collected from study participants for laboratory analysis for the evaluation of salivary alpha amylase level. Selection of participants: Based on inclusion and exclusion criteria age and gender matched total 160 study subjects (age- 30 to 60 years) were entailed in this study. Inclusion criteria: The individuals must be systemically healthy, age group between 30-60 years, control

group with a gingival index  $\leq 1$  and no attachment loss, gingivitis group participants with sulcular bleeding index and gingival index  $\geq 2$ , in localized chronic periodontitis group participants having sulcular bleeding index and gingival index  $\geq 2$  and clinical attachment loss in  $\leq 30\%$  of sites, in generalized chronic periodontitis with sulcular bleeding index and gingival index score  $\geq 2$  and clinical attachment loss in  $\geq 30\%$  of sites. Exclusion criteria: Those who had cardiovascular, respiratory diseases, diabetes mellitus, systemic inflammatory conditions, immunodeficiency, pregnancy, and breast feeding, have taken antibiotics not beyond past six months, smokers, undergone periodontal treatment not more than past six months, history of salivary gland disorder, and any other oral infections like Herpes and Candida.

### **Aim**

The aim of this study is to evaluate the salivary alpha amylase level as an inflammatory biomarker in chronic periodontal disease patients.

### **Study Method**

Sample size: Total 160 (30-60 years of age) individuals were categorized into four groups, on the basis of inclusion and exclusion criteria. Group I (Control group) - consisting of 40 subjects with healthy periodontium (20 males and 20 females). Group II (Study group)- consisting of 40 subjects (20 males and 20 females) with generalized chronic gingivitis. Group III (Study group)- consisting of 40 subjects (20 males and 20 females) with localized chronic periodontitis. Group IV (Study group)- consisting of 40 subjects (20 males and 20 females) with generalized chronic periodontitis. Statistics were applied to the collected data. The obtained values were tabulated, and the means and standard deviations of each parameter measured were computed. Total sample size required  $n=160$  as per standard calculations. Before enrolling into the study, the following clinical parameters were recorded in study participants of all the groups:

#### **Clinical Parameters**

1. Oral hygiene Index-Simplified (OHI-S) (Green and Vermilion in 1964)
2. Sulcular Bleeding Index (SBI) (Muhleman H.R. in 1971)
3. Gingival Index (GI) (Loe and Silness in 1963)
4. Probing Pocket Depth (PPD)
5. Clinical Attachment Level (CAL)

The following salivary biomarkers were assessed by collecting unstimulated saliva from study participants of all the groups:

**Salivary Biomarkers:** Salivary Alpha Amylase level.

#### **Saliva Collection And Biochemical Analysis:<sup>7</sup>**

It was requested of the participants not to brush their teeth, have a meal, or consume any liquid for an hour before collecting saliva. The patients were made to sit down between the hours of 11 a.m. and 12 p.m. and were asked to collect saliva in their mouths and then expectorate into the container without stimulation, as shown in Figs.

1.1 and 1.2, in order to avoid diurnal variation. The samples were taken for collection right away and examined biochemically. **Estimation of Salivary Alpha Amylase by Method:** CNP-G3, kinetic assay method, **Model:** Semi auto analyzer (ROBONIC), **Kit:** Erba Mannheim Amylase Kit. Colorimetric analysis was used to determine the amylase activity in saliva.

#### Procedure

Amylase estimation was done by Kinetic method. 25  $\mu$ l of saliva were collected, centrifuged for 15 minutes at 3000 rpm, and then 1000  $\mu$ l of the reagent (the amylase kit) were combined and incubated at 37degree centigrade. It was timed at 15 seconds and the absorbance at 405nm was recorded. At 30-second intervals, two more absorbance measurements were taken. The change in mean absorbance per minute was calculated, and factor 4824 was then multiplied. To perform this test, 500 $\mu$ l of amylase mono-reagent was taken in glass tube with the help of calibrated micropipette. 400 $\mu$ l of salivary amylase reagent, R1 and 100 $\mu$ L of amylase reagent, R2, pipetted in same test tube and mixed properly. The presence of amylase in this reaction caused the color to be lemon yellow. An auto-analyzer was used to measure the salivary amylase level after a 60-second waiting period, as shown in Figs. 1.3 to 1.8.



**Fig 1.1:** Collection of unstimulated saliva



**Fig 1.2:** Container with saliva sample



**Fig 1.3:** Saliva drawn into pipette and placed in test tube



**Fig 1.4:** Saliva sample subjected to centrifugation at 3000 rpm for 15 minutes



**Fig 1.5:** Amylase Reagent loaded and mixed with saliva sample



**Fig 1.6:** Colour change observed after adding reagent



**Fig 1.7:** The resultant saliva sample has been subjected to auto- analyzer



**Fig 1.8:** The readings were recorded from digital display

## Results

The saliva samples were collected from study participants for laboratory analysis to evaluate salivary alpha amylase concentration. Data collected were statistically analyzed using statistical package SPSS 22.0 (SPSS Inc., Chicago, IL) and level of significance was set at  $p < 0.001$ . Descriptive statistics was performed to assess the mean  $\pm$  standard deviation of the respective groups. Inferential statistics to find out the association between the groups was done using one way ANOVA test, post hoc tukey test and independent t-test. A one-way ANOVA uses the following null and alternative hypotheses:  $H_0$  (null hypothesis):  $\mu_1 = \mu_2 = \mu_3 = \dots = \mu_k$  (all the population means are equal)  $H_1$  (alternative hypothesis): at least one population mean is different from the rest.

### Demographic details of study participants of all the four groups.

Table 1 shows the demographic details of study participants in each group. Mean age of healthy subjects (Group I) was  $31.05 \pm 0.85$  years. Mean age of subjects with chronic generalized gingivitis (Group II) was  $35.55 \pm 3.55$  years. Mean age of subjects with localized chronic periodontitis (Group III) was  $42.65 \pm 6.50$  years. Mean age of subjects with generalized chronic periodontitis (Group IV) was  $49.00 \pm 5.95$  years. There were equal number of males and females in each group. The male – female proportion of the study cases in group I, group II, group III, and group IV are same i.e., 50% respectively.

### Comparison of OHI-S among four groups

Table 2 shows the comparison of OHI-S score between four study groups. Mean OHI-S score of healthy subjects (Group I) was  $0.05 \pm 0.22$ . Mean OHI-S score of subjects with chronic generalized gingivitis (Group II) was  $1.53 \pm 0.51$ . Mean OHI-S score of subjects with localized chronic periodontitis (Group III) was  $1.85 \pm 0.36$ . Mean OHI-S score of subjects with generalized chronic periodontitis (Group IV) was  $2.00 \pm 0.00$ . Mean OHI-S score was higher among subjects with generalized chronic periodontitis followed by subjects with localized chronic periodontitis and generalized chronic gingivitis. Least OHI-S score was seen in healthy subjects. This difference in OHI-S among four groups was significant ( $p < 0.001$ ).

### **Comparison of Sulcular Bleeding Index (SBI) among four groups**

Table 3, shows the comparison of sulcular bleeding index between all study groups. Mean sulcular bleeding index of healthy subjects (Group I) was  $0.00 \pm 0.00$ . Mean sulcular bleeding index of subjects with generalized chronic gingivitis (Group II) was  $2.93 \pm 0.76$ . Mean sulcular bleeding index of subjects with localized chronic periodontitis (Group III) was  $3.95 \pm 0.78$ . Mean sulcular bleeding index of subjects with generalized chronic periodontitis (Group IV) was  $4.70 \pm 0.46$ . Mean sulcular bleeding index was higher among subjects with generalized chronic periodontitis followed by subjects with localized chronic periodontitis and generalized chronic gingivitis. Least sulcular bleeding index score was seen in healthy subjects. This difference in sulcular bleeding index among four groups was significant ( $p < 0.001$ ).

### **Comparison of Gingival Index among four groups**

Table 4, shows the comparison of gingival index between all study groups. Mean gingival index of healthy subjects (Group I) was  $0.00 \pm 0.00$ . Mean gingival index of subjects with generalized chronic gingivitis (Group II) was  $2.18 \pm 0.38$ . Mean gingival index of subjects with localized chronic periodontitis (Group III) was  $2.35 \pm 0.66$ . Mean gingival index of subjects with generalized chronic periodontitis (Group IV) was  $2.45 \pm 0.50$ . Mean gingival index score was higher among subjects with generalized chronic periodontitis followed by subjects with localized chronic periodontitis and generalized chronic gingivitis. Least gingival score was seen in healthy subjects. This difference in gingival index among four groups was significant ( $p < 0.001$ ).

### **Comparison of Probing Pocket Depth among four groups**

Table 5, shows the comparison of pocket probing depth score between all study groups. Mean pocket probing depth of healthy subjects was  $0.00 \pm 0.00$ . Mean pocket probing depth of subjects with generalized chronic gingivitis (Group II) was  $0.00 \pm 0.00$ . Mean pocket probing depth of subjects with localized chronic periodontitis (Gr III) was  $6.55 \pm 0.50$ . Mean pocket probing depth of subjects with generalized chronic periodontitis (Gr IV) was  $7.40 \pm 0.74$ . Mean pocket probing depth was higher among subjects with generalized chronic periodontitis followed by subjects with localized chronic periodontitis and generalized chronic gingivitis. Least of pocket probing depth score was seen in healthy subjects. This difference in pocket probing depth among four groups was significant ( $p < 0.001$ ).

### **Comparison of Clinical Attachment Level among four groups**

Table 6, shows the comparison of CAL score between all study groups. Mean CAL score of healthy subjects (Group I) was  $0.00 \pm 0.00$ . Mean CAL score of subjects with generalized chronic gingivitis (Group II) was  $0.00 \pm 0.00$ . Mean CAL score of subjects with localized chronic periodontitis (Group III) was  $3.70 \pm 1.14$ . Mean CAL score of subjects with generalized chronic periodontitis (Group IV) was  $4.75 \pm 0.44$ . Mean CAL score was higher among subjects with generalized chronic periodontitis followed by subjects with localized chronic periodontitis and generalized chronic gingivitis. Least CAL score was seen in healthy subjects. This difference in CAL among four groups was significant ( $p < 0.001$ ).



### **Comparison of Salivary Alpha Amylase Level (U/mL) among four groups**

Table 7, shows the comparison of Salivary amylase level (U/mL) between all study groups. Mean salivary amylase level of healthy subjects was  $70.29 \pm 1.91$ . Mean salivary amylase level of subjects with generalized chronic gingivitis (Group II) were  $88.60 \pm 1.62$ . Mean salivary amylase level of subjects with localized chronic periodontitis (Group III) was  $118.02 \pm 1.54$ . Mean salivary amylase level of subjects with generalized chronic periodontitis (Group IV) was  $125.80 \pm 1.62$ . Mean salivary amylase level was higher among subjects with generalized chronic periodontitis followed by subjects with localized chronic periodontitis and generalized chronic gingivitis. Least of salivary amylase level (U/mL) score was seen in healthy subjects. This difference in pocket Salivary amylase level (U/mL) four groups was significant ( $p < 0.001$ ).

### **Comparison of variables between males and females in each group**

Table 8 reveals comparison of each variable between males and females in each group. It showed that there was no statistically significant difference in OHI-S score between males and females in healthy group, generalized chronic gingivitis, localized chronic periodontitis, and generalized chronic periodontitis groups having p value 0.154, 0.119 and 1.000 respectively. It showed that there was no statistically significant difference in sulcular bleeding index score between males and females in healthy group, and generalized chronic gingivitis group; however, females of localized chronic periodontitis group ( $4.35 \pm 0.81$ ) and generalized chronic periodontitis group ( $4.80 \pm 0.37$ ) were shown significantly higher sulcular bleeding index as compared to males of respective groups. It showed that there was no statistically significant difference in gingival index score between males and females in healthy group, and subjects with localized chronic periodontitis group; however, females of generalized chronic gingivitis group ( $2.35 \pm 0.49$ ) and generalized chronic periodontitis ( $2.25 \pm 0.44$ ) group showed significantly higher gingival index as compared to males of respective groups. Table also showed that there was no statistically significant difference in probing pocket depth between males and females in healthy group, and generalized chronic gingivitis group; however, females of localized chronic periodontitis group ( $6.80 \pm 0.41$ ) and generalized chronic periodontitis group ( $7.80 \pm 0.41$ ) showed significantly higher pocket probing depth as compared to males of respective groups. It showed that there was no statistically significant difference in clinical attachment level score between males and females in healthy group, and generalized chronic gingivitis group; however, females of localized chronic periodontitis group ( $4.35 \pm 0.49$ ) and generalized chronic periodontitis group ( $4.90 \pm 0.31$ ) showed significantly higher CAL score as compared to males of respective groups. Table also showed statistically significant difference in salivary amylase level, females in each group showed significantly higher Salivary amylase levels as compared to males of respective groups ( $p < 0.001$ ).

**Table 1:** Demographic Details of Study Participants of All The Four Groups

Variable	Category	Healthy (Gr-I)	GCG (Gr-II)	LCP (Gr-III)	GCP (Gr-IV)
Age	--	31.05 ± 0.85	35.55 ± 3.55	42.65 ± 6.50	49.00 ± 5.95
Gender	Male	20 (50%)	20 (50%)	20 (50%)	20 (50%)
	Female	20 (50%)	20 (50%)	20 (50%)	20 (50%)

**Table 2:** Comparison of Ohi-S Among Four Groups

Groups	Mean	SD	F value	p value
Healthy (Gr I)	0.05	0.22	293.218	<0.001*
Generalized chronic gingivitis (Gr II)	1.53	0.51		
Localized chronic periodontitis (Gr III)	1.85	0.36		
Generalized chronic periodontitis (Gr IV)	2.00	0.00		

**Table 3:** Comparison of Sulcular Bleeding Index (Sbi) Among Four Groups

Groups	Mean	SD	F value	p value
Healthy (Gr-I)	0.00	0.00	481.637	<0.001*
Generalized chronic gingivitis (Gr-II)	2.93	0.76		
Localized chronic periodontitis (Gr-III)	3.95	0.78		
Generalized chronic periodontitis (Gr-IV)	4.70	0.46		

**Table 4:** Comparison of Gingival Index Among Four Groups

Groups	Mean	SD	F value	p value
Healthy (Gr-I)	0.00	0.00	259.752	<0.001*
Generalized chronic gingivitis (Gr-II)	2.18	0.38		
Localized chronic periodontitis (Gr-III)	2.35	0.66		
Generalized chronic periodontitis (Gr-IV)	2.45	0.50		

**Table 5:** Comparison of probing pocket depth among four groups

Groups	Mean	SD	F value	p value
Healthy (Gr-I)	0.00	0.00	3236.340	<0.001*
Generalized chronic gingivitis (Gr-II)	0.00	0.00		
Localized chronic periodontitis (Gr-III)	6.55	0.50		
Generalized chronic periodontitis (Gr-IV)	7.40	0.74		

**Table 6:** Comparison of clinical attachment level among four groups

Groups	Mean	SD	F value	p value
Healthy (Gr-I)	0.00	0.00	661.069	<0.001*
Generalized chronic gingivitis (Gr-II)	0.00	0.00		
Localized chronic periodontitis (Gr-III)	3.70	1.14		
Generalized chronic periodontitis (Gr-IV)	4.75	0.44		

**Table 7:** Comparison of Salivary Alpha Amylase Level (U/Ml) Among Four Groups

Groups	Mean	SD	F value	p value
Healthy (Gr-I)	70.29	1.91	281.419	<0.001*
Generalized chronic gingivitis (Gr-II)	88.60	1.62		
Localized chronic periodontitis (Gr-III)	118.02	1.54		
Generalized chronic periodontitis (Gr-IV)	125.80	1.62		

**Table 8:** Comparison of Variables Between Males And Females In Each Group

Variable	Gender	Healthy		GCG		LCP		GCP	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
OHI-S	Male	0.00	0.00	1.40	0.50	1.85	0.36	2.00	0.00
	Female	0.10	0.31	1.65	0.49	1.85	0.36	2.00	0.00
	p value	0.154		0.119		1.000		--	
SBI	Male	0.00	0.00	2.80	0.70	3.55	0.51	4.60	0.51
	Female	0.00	0.00	3.05	0.83	4.35	0.81	4.80	0.37
	p value	--		0.307		0.001*		0.040*	
GI	Male	0.00	0.00	2.00	0.00	2.25	0.72	2.65	0.49
	Female	0.00	0.00	2.35	0.49	2.45	0.61	2.25	0.44
	p value	--		0.005*		0.346		0.010*	
PPD	Male	0.00	0.00	0.00	0.00	6.30	0.47	7.00	0.79
	Female	0.00	0.00	0.00	0.00	6.80	0.41	7.80	0.41
	p value	--		--		0.001*		<0.001*	
CAL	Male	0.00	0.00	0.00	0.00	3.05	1.23	4.60	0.50
	Female	0.00	0.00	0.00	0.00	4.35	0.49	4.90	0.31
	p value	--		--		<0.001*		<0.030*	
Salivary amylase	Male	68.69	0.89	87.16	0.58	116.65	0.51	124.36	0.57
	Female	71.89	1.13	90.04	0.81	119.40	0.81	127.24	0.82
	p value	<0.001*		<0.001*		<0.001*		<0.001*	

## Discussion

Dissolution of polysaccharide –1-4 glycosidic bonds is catalyzed by salivary amylase, an enzyme from the hydrolase family. The hydrolysis of dietary starch to produce maltose and then glucose is aided by the calcium-containing enzyme amylase. The lactic acid-producing bacteria that reside on the surface of plaque on teeth will always have access to glucose as a result. This ultimately lowers pH and causes issues with oral health.<sup>8</sup> According to the findings of our study, subjects with generalized chronic periodontitis ( $2.00 \pm 0.00$ ) had a higher mean OHI-S score than those with localized chronic periodontitis ( $1.85 \pm 0.36$ ) or generalized chronic gingivitis ( $1.53 \pm 0.51$ ). Healthy subjects had the lowest OHI-S score ( $0.05 \pm 0.22$ ). Generalized chronic periodontitis ( $4.70 \pm 0.46$ ), localized chronic periodontitis ( $3.95 \pm 0.78$ ), and generalized chronic gingivitis ( $2.93 \pm 0.76$ ), were associated with a significant increase in the mean sulcular bleeding index. Healthy subjects had the lowest sulcular bleeding index score ( $0.00 \pm 0.00$ ). Our study's findings showed that subjects with generalized chronic periodontitis ( $2.45 \pm 0.50$ ) had higher mean gingival index scores

than those with localized chronic periodontitis ( $2.35 \pm 0.66$ ) or generalized chronic gingivitis ( $2.18 \pm 0.38$ ). Healthy subjects had the lowest gingival index score ( $0.00 \pm 0.00$ ). Generalized chronic periodontitis had the greatest increase in mean pocket probing depth ( $7.40 \pm 0.74$ ), followed by subjects with localized chronic periodontitis ( $6.55 \pm 0.50$ ), and generalized chronic gingivitis had the least increase in mean pocket probing depth ( $0.00 \pm 0.00$ ) and healthy subjects had the least increase in mean pocket probing depth ( $0.00 \pm 0.00$ ). The mean CAL score increased significantly with generalized chronic periodontitis ( $4.75 \pm 0.44$ ), localized chronic periodontitis ( $3.70 \pm 1.14$ ) and healthy subjects ( $0.00 \pm 0.00$ ). The mean CAL score decreased significantly with generalized chronic gingivitis ( $0.00 \pm 0.00$ ) and healthy subjects ( $0.00 \pm 0.00$ ).

According to the findings of our study, generalized chronic periodontitis has higher levels of salivary alpha amylase than localized chronic periodontitis, generalized chronic gingivitis, and periodontally healthy subjects, respectively. The mean salivary alpha amylase level increased statistically significantly from group I to group IV when compared between all the groups ( $P < 0.001$ ). With generalized chronic periodontitis ( $125.80 \pm 1.62$ ), generalized chronic gingivitis ( $88.60 \pm 1.62$ ), and localized chronic periodontitis ( $118.02 \pm 1.54$ ), a rise was observed in the level of mean salivary amylase. Healthy subjects had the lowest salivary amylase level (U/mL) score ( $70.29 \pm 1.91$ ). This is in line with research done in 2010 by Goncalves LR *et al.* that showed higher SAA levels in chronic periodontitis.<sup>9</sup> In 2011, Sanchez GA, Miozza V, Delgado A, and Busch L discovered that severe periodontitis had higher levels of SAA than moderate periodontitis and healthy subjects.<sup>10</sup> Another study by Hyun CK, Hyung SK, Kyung SM, and Nam PC in 2010 compared subjects with healthy, gingivitis, and chronic periodontitis for salivary amylase level, discovered that chronic periodontitis had a higher SAA level than gingivitis and subjects in good health.<sup>11</sup> In a 2014 study, Kejriwal S, Bhandary R, Thomas B, and Kumari S measured SAA level using the kinetic assay method and discovered that periodontitis patients had significantly higher values than gingivitis patients and healthy participants.<sup>12</sup> The literature provided three justifications for the findings that periodontal diseases increase SAA levels. First off, the elevated levels could be a result of salivary glands producing and secreting more amylase in response to inflammatory conditions like gingivitis and periodontitis to strengthen the oral defence mechanism.<sup>13</sup> Second, studies have shown that *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* use amylase as a key lipopolysaccharide binding protein to inhibit bacterial adhesion and biofilm formation.<sup>14</sup> This suggests that salivary amylase is an essential defense molecule for the oral cavity's innate immunity given that it appears to have a high concentration.<sup>15</sup> Not to mention, the increased levels may also be partly explained by an increase in plasma protein leakage into saliva as a result of inflammation.<sup>16</sup> Additionally, subjects with similar gender and age have been used. The results showed that the OHI-S scores were identical between males and females in each group according to gender. It was found that in the groups with generalized chronic gingivitis and chronic periodontitis, women had significantly higher gingival indices than men. The sulcular bleeding index, PPD, and CAL of the generalized and localized chronic periodontitis groups in women were

more as compared to men. Salivary alpha amylase levels in females have been found to be slightly higher than those in males. This is in line with the results of a research conducted in 2009 by Gallo CD, Mimura MA, and Sugaya NN, which showed that salivary amylase levels were more in women than in men due to women's greater susceptibility to stressors. Salivary amylase levels in women significantly increased both before and after stress. Emotional expression can cause oral diseases directly or indirectly through psychological changes. The mechanisms of the oral mucosal response to exogenous or endogenous antigens become dysregulated in response to psychological stress. Due to the proliferation of TCD8+ lymphocytes, which act as cytotoxic reaction mediators and result in oro-mucosal alterations, TCD4+ cell imbalance is more prevalent in women.<sup>17</sup> Salivary alpha amylase showed a significant positive correlation in our study, suggesting that they may be involved in the host response.

### **Conclusion**

It has been observed that salivary alpha amylase levels were more in individuals with chronic periodontal disease as compared to periodontally healthy subjects. Additionally, generalized chronic periodontitis was found to have higher salivary alpha amylase levels than localized chronic periodontitis and generalized chronic gingivitis. Salivary alpha amylase levels were slightly more in female subjects than in males. It follows that salivary alpha amylase level concentration of inflammatory biomarkers is a safer method that can be used in the diagnosis and prognosis of chronic periodontal disease.

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