Evaluation of Salivary α- Amylase Enzyme Activity in Smoker and Peptic Ulcer Patients affected by Periodontitis in Relation to Clinical Periodontal Parameters

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Abstract

Objectives: To investigate the activity of salivary α-amylase (SA) in subject affected by periodontitis alone or associated with peptic ulcer disease (PUD) with or without smoking.

Methods: The study population was divided into control group (C), study groups; periodontitis (G1), periodontitis+ PUD+ smokers (G2), periodontitis+ PUD+ nonsmoker (G3). The clinical periodontal parameters including plaque index (PI), gingival index (GI), probing pocket depth (PPD), and clinical attachment level (CAL) were recorded. In addition, unstimulated whole saliva was collected from these patients for analysis of SA level.

Results: Analysis showed that PI and GI showed significant difference among study groups versus controls. G2 exhibited significantly higher PPD and CAL as compared with G1 but not with G3. For SA activity, G2 and G3 showed significantly higher activity as compared with C and G1 groups. Yet, no significant difference was seen between C and G1 and between G2 and G3. Activity of SA was positively and significantly ($P < 0.05$) correlated with increasing PPD in all study groups and with CAL measurements of G2 only.

Conclusions: The results suggested that SA could be used as a marker for the diagnosis of PUD in subjects affected by periodontitis with or without smoking.

Keywords: *periodontitis, diagnosis of PUD ; peptic ulcer, alpha-Amylases, toxicity;*

Introduction

Traditionally, diagnosis of periodontal diseases (PD) is performed clinically together with radiographic assessment, which often associated with possibility of errors in measurements. Alteration in the level of different markers in the oral fluids during the course of PD may represent a valuable diagnostic tool to assess the severity and susceptible individuals[1].

Saliva contains range of proteins, actively involved in the innate immune response, including salivary amylase, lysozyme, cystatins, and mucins[2]. These molecules form a dynamic defensive network by synergistic/cumulative effects contributing to immune system activation/ modulation[2, 3]. Among these proteins, salivary α-amylase, well-known for its inhibitory activity against range of microorganisms and significantly increased during periodontitis[1]. Smoking is a well-documented risk factor that negatively influencing the periodontal health by impairing immune response associated with increased severity of PD[4]. Previous studies suggested that smoking inhibits salivary amylase by the interaction of one of the smoking product, unsaturated aldehydes, with -SH group of the enzyme molecule[5].

PD were suggested to be related to different systemic diseases affecting the human body. Peptic ulcer disease (PUD) which is a condition affecting the integrity of gastrointestinal system. The etiology of PUD is mainly attributed to Gram-negative *Helicobacter pylori* (*H. pylori*)[6]. Interestingly, dental biofilm could be a reservoir for this bacterium from which infection of stomach may occur[7]. Intense plaque control measures

were associated with less recurrence of PUD[7]. Further support was obtained from other studies that indicated a potential relationship of PD with PUD[8]. Comparison of salivary enzymes between healthy subjects and patients with PUD showed that the latter group was associated with higher α-amylase activity compared to the control[9]. One of major risk factors for PUD is smoking which not only induce ulceration and increase acidity of the stomach but it was suggested as a predisposing factor for *H. pylori* gastric ulceration[10].

The aim of this cross-sectional study was to evaluate the activity of salivary α-amylase (SA) in subject with periodontitis alone or associated with PUD and/or cigarette smoking habit. In addition, correlation of amylase activity was determined in relation to range of clinical periodontal parameters.

Methods

Study design

This was a prospective cross-sectional study which was conducted in College of Dentistry, University of Baghdad. The study was started at Feb to Jun 2019 after obtaining approval from ethics committee in the college in accordance with Tokyo and Helsinki declaration for humans.

Subjects included in this study were randomly recruited from patients attending the Department of Periodontics. For patient affected with periodontitis, history was taken for each patient to record the age, cigarette smoking status, medical history. Diagnosis whether they currently having PUD or not was based on self-reporting by the patients or via the drugs they are taken. Subjects with healthy periodontium, nonsmoker, and not suffering from PUD were designated as control (C) group and were included mainly to obtain baseline data for the normal activity level of salivary α–amylase. Other patients (study groups), were divided into: G1 patients with periodontitis, no PUD and nonsmokers, G2 included patients with periodontitis, PUD, and smokers, and G3 patients with periodontitis, PUD and nonsmokers. The exclusion criteria were females, other systemic conditions, use of mouthwashes, under chemotherapy, radiation therapy, or medications that cause xerostomia. The aim and flow of the study were explained for the participants, who signed a consent form prior to the study.

Clinical parameters and examiners' calibrations

Periodontitis was defined as the presence of teeth with probing pocket depth (PPD) ≥4mm with clinical attachment loss, this made according to the international classification system for PD[11]. The clinical periodontal parameters which include plaque index (PI)[12], gingival index (GI)[13], bleeding on probing (BOP), PPD and clinical attachment level (CAL) were recorded for each patient. Measurements were made at four sites per tooth using William's periodontal probe. Before starting the study, inter- and intra-examiner calibration sessions were conducted among the clinicians. The readings among them were considered consistent when weighed κ values were ≥ 0.7 at 95% confidence interval was reached for each index.

Saliva collection and α-amylase activity determination

Unstimulated whole saliva was collected for analysis, before that the patient was instructed to rinse his mouth several times by water and then wait for (1-2min) for water clearance. After collecting the sample, it was centrifuged at (4000rpm) for (10min), then the samples were frozen at (-20^{o}C) until α–amylase analysis was done. Salivary α–amylase analysis done by using α–amylase liquicolor (colorimetric test for α–amylase) (Demeditec Diagnostics GmbH, Kiel, Germany) following manufacturer's instructions manual.

Statistical Analysis

Descriptive analysis was performed in the form of means, standard deviation (SD). Since the data were normally distributed then inferential statistics was conducted by using one-way ANOVA test and Tukey's test. Correlation coefficient (r) for α-amylase activity with clinical parameters was calculated by using Pearson correlation test. Significant level was considered at $p<0.05$.

Results

Recruitment of patients was illustrated in Figure 1. Demographic characteristics and distribution of the patients were shown in Table 1, while clinical parameters were summarized in table 2.

Figure 1 Flow diagram of the study

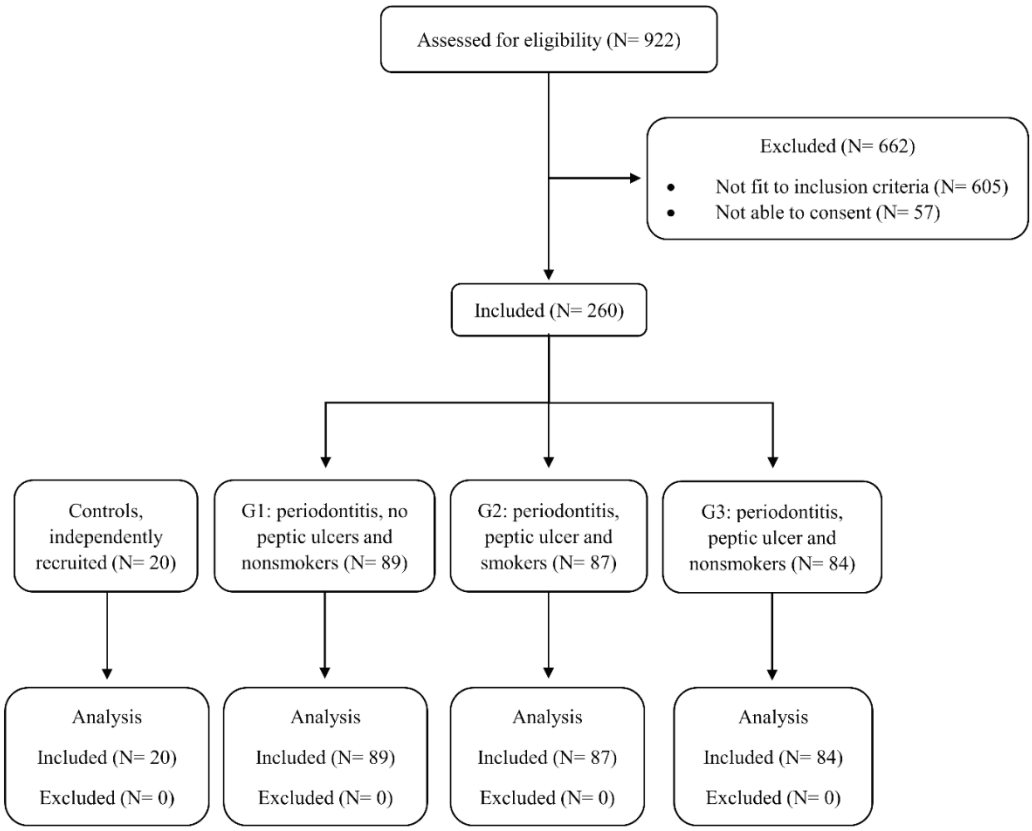

Table 1: Characteristics of the study population

Age range	**29-73**
Average age (mean± SD)	51.1± 11.4
Groups¶	N (%)
Control	
C	20 (100)
Study	
G1	478 (69.1)
G2	82 (11.8)
G3	132 (19.1)
Total	692 (100)

¶ C1: control group, nonsmokers and no peptic ulcer, G1: periodontitis, no PUD and nonsmokers, G2: periodontitis, PUD and smokers, G3: periodontitis, PUD and nonsmokers

Table 2: Clinical parameters of all groups

	Mean± SD			
Groups	**PI**	**GI**	**PPD**	**CAL**
C	0.53± 0.36	0.79± 0.41		
G1	2.08± 0.53	2.04± 0.72	4.73± 0.96	4.72± 1.33
G2	2.28± 0.55	2.27± 0.53	6.31± 1.58	5.91± 1.42
G3	2.48± 0.37	2.36± 0.47	5.27± 1.53	5.70± 1.33

Analysis showed that PI and GI did not show any significant difference among the groups. G2 patients exhibited significantly deeper PPD as compared to G1 but not with G3. The same pattern was observed in association with CAL measurements in which significant difference was observed between G2 and G1 only (Table 3).

Table 3: Comparison of clinical parameters among all groups

Comparisons	**p value***			
	PI	**GI**	**PPD**	**CAL**
C vs G1	S	S		
C vs G2	S	S		
C vs G3	S	S		
G1 vs G2	NS	NS	NS	NS
G1 vs G3	NS	NS	S	S
G2 vs G3	NS	NS	NS	NS

* p value<0.05 by ANOVA test, S= significant, NS= non-significant

For SA activity, G2 and G3 showed significantly higher activity as compared with C group and G1. However, no significant difference was seen between C and G1. No significant difference was shown between G2 and G3 (Table 4). Activity of salivary amylase enzyme was significantly and positively correlated with PI in G2 and G3 groups only. While PPD was significantly and positively related to the enzyme activity in all groups which showed moderate-strong correlation. However, only CAL in G2 group was significantly increased with increased activity of SA (Table 5).

Table 4: Comparison of salivary α-amylase activity among all groups

Groups	α-amylase ± SD	Comparison
	U/l[§]	
C	39.14± 27.03	C vs G1
G1	72.43± 31.80	C vs G2*
G2	154.1± 29.60	C vs G3*
G3	121.8± 55.50	G1 vs G2*
		G1 vs G3*
		G2 vs G3

§ Activity of α-amylase (ΔA/min)

* Significant at p<0.05 by ANOVA test

Table 5: Relation of α-amylase activity with clinical parameters in all groups

Groups	PI	GI	PPD	CAL
C	0.280	0.119		
G1	0.414	0.417	0.644*	0.331
G2	0.606*	0.249	0.508*	0.567*
G3	0.777*	0.183	0.726*	0.448

* Significant correlation at p<0.05 by using Pearson test

Discussion

Salivary amylase is a calcium containing metalloenzyme responsible for initial cleavage of α-glycosidic bonding of glucose-polymers such as starch, glycogen and dextrin[14]. Salivary proteins play important role in the innate immune response to PD[2]. Interestingly, presence of α-amylase was detected in the acquired pellicle suggesting a role in the adhesion of primary colonizers to tooth surface and it may supply these microorganisms with the needed glucose due its digestive action[15].

PUD could be developed due to several risk factors such as *H. pylori* infection, smoking and alcohol consumption. Recent studies have shown that the *H. pylori* which is associated with chronic gastritis, peptic ulcer, and gastric malignancies, is also present in the dental plaque biofilm[16]. Literatures have suggested a positive association between peptic ulceration and periodontal pathogens. Results from meta-analysis have shown that combining periodontal therapy with anti-*H. pylori* medications have a positive impact on the prognosis of PUD[17].

In this study, analysis of clinical parameters showed that PI and GI did not show any significant difference among the study groups. Both smoker and nonsmoker patients with peptic ulcer exhibited significantly deeper periodontal pocket as compared to nonsmoker and no PUD patients. This agrees with other study which indicated that subjects with deeper pockets and alveolar bone loss were more prone to develop PUD independent from other risk factor[18]. Further, number of missing teeth due to PD was reported to be significantly increased in association with increased prevalence of gastric ulcer[19]. Nevertheless, results from another matched case-control study did not show presence of such association between the two conditions[8]. This conflict could be due to that subjects in both control and study groups in the latter study were infected with *H. pylori*. Also, a study conducted by Khader et al. (2003) did not show any significant association between PUD and increased PPD; however, significant association was observed in relation to CAL[20], which agree with results of the current survey that showed same results in regard to CAL measurements.

SA activity in this study was significantly upregulated in patients with periodontitis affected by PUD as compared to healthy controls or those affected by periodontitis only. This agrees with previous study which showed significant increase in SA concentration in association with periodontitis patients as compared to healthy individuals[21]. Increased secretion of SA and other acinar proteins of glandular origin was attributed to defensive response of salivary glands to increased population of periodontitis-associated bacteria[22]. In addition, adherence and biofilm formation of Gram-

negative bacteria was shown to be disrupted by amylase activity by its affinity to binding to the major virulence factor, lipopolysaccharide[23]. Henskens and co-authors have proposed that upregulation in the level of salivary proteins during periodontitis could be due to increased leakage of plasma proteins into saliva in response to inflammation[24]. Activity of SA is negatively affected by smoking which is associated with increased reactive nitrogen species that interact with -SH active sites of the enzyme[25;26]. However, no significant difference was observed in the amylase activity between G2 and G3 in our study. This may suggest that the stimulatory effect of periodontal pathogens overwhelmed the inhibitory effect of smoking product on the enzyme activity. Further support for the role of α-amylase activity during the course of PD was observed in the positive correlation between the enzyme and the increase of PPD in all patients affected with periodontitis, while this relation was also shown in association with CAL in smoker patients affected with peptic ulcer. Previous study has indicated a potential role of SA in enhancing adhesion of the primary colonizers to the acquired pellicle[15]. Interestingly, score of PI showed positive relation with increased activity of α-amylase in periodontitis patients suffering from peptic ulcer whether smoking or not.

There are limitations to this study such as data about *H. pylori* infection and NSAID use were not available in this study. In addition, frequency and duration of smoking were not detected which need to be further investigated in relation to the activity of salivary enzymes activity.

Conclusions

Within limits, results from our study indicated higher activity of SA in patients with periodontitis together with peptic ulcer and this activity increased in association with smoking. This suggests that periodontitis with smoking act synergistically as risk factors for development of PUD. In addition, increased activity of this enzyme could enhance dental plaque accumulation and increased PPD. Thus, SA in smoker patients affected by periodontitis could be used as predictive and prognostic tool for PUD.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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