Assessment of Salivary α-amylase and Flow Rate Levels and Their Correlation with Gingivitis and Severity of Chronic Periodontitis (Part: 1)

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ABSTRACT

Background: Periodontal diseases are bacterial infections of the gingiva, bone and attachment fibers that support the teeth and hold them in the jaw. α-amylase is an enzyme, produced mainly by parotid gland and it seems to play a role in maintaining mucosal immunity.

Aims of the study: Determine the salivary levels of α-Amylase and flow rate and their correlations with clinical periodontal parameters(Plaque Index , Gingival Index , Bleeding on Probing , Probing Pocket Depth , and Clinical Attachment Level) and the correlation between α-Amylase with flow rate of study groups that consist of (patients had gingivitis and patients had chronic periodontitis with different severities(mild ,moderate ,severe) and control group .

Materials and Methods: Salivary α-Amylase and flow rate levels with clinical periodontal parameters(Plaque Index , Gingival Index , Bleeding on Probing , Probing Pocket Depth , and Clinical Attachment Level) were measured from 75 males , age ranged (30-45) years old, that divided into study groups(group of 45chronic periodontitis patients with different severities which sub-grouped into (Mild=15, Moderate=15 and Severe=15), group of 15 patients with gingivitis) and control group comprised 15 subjects had clinically healthy periodontium.

Results: The levels of salivary α-amylase in patients had chronic periodontitis were the highest followed by patients had gingivitis .Highly significant differences were demonstrated between each pairs of chronic periodontitis subgroups hence, the highest level at severe chronic periodontitis subgroup patients. Flow rate decreased in gingivitis group and chronic periodontitis with its different severities. Highly significant strong positive correlations were found between a-amylase with clinical periodontal parameters at all groups and subgroups.

Conclusions: The findings of the present study suggest that salivary a-Amylase can help to monitor the progression of the periodontal disease.

Keywords: gingivitis, chronic periodontitis, α-amylase, saliva, flow rate. **(J Bagh Coll Dentistry 2016; 28(4):115-121)**

INTRODUCTION

Periodontal diseases (PD) are bacterial infections of the gingiva, bone and attachment fibers that support the teeth and hold them in the jaw [(1)] . The two common forms of periodontal diseases are gingivitis and periodontitis. Gingivitis is a reversible inflammatory condition of the soft tissue surrounding the teeth (the gingiva) without the involvement of the attachment apparatus, whereas periodontitis involves the deeper periodontium resulting in the clinical attachment loss with the destruction of gingiva, periodontal ligament, cementum and alveolar bone [(2,3)]. Regrettably, the resulting tissue damage is irreversible and it is usually asymptomatic until teeth become loose [(4,5)].

Chronic periodontitis (CP) is very common disease and it is generally a slowly progressing form of PD, but may have periods of rapid progression [(6,7)].

Saliva is a unique complex, important body fluid [(8-10)]. Salivary sample, since it is a simple, non-invasive and safer method, besides; its storage is simple and cost-efficient. Saliva contains locally produced microbial and host response mediators, as well as, systemic (serum) markers [(11)].

Salivary flow rate (FR), it is the amount of saliva naturally produced by the salivary glands. In adults, normal total (FR) UP to 3 ml/min [(12-14)] hence, decreased in patients with CP [(15)].

The α-Amylase is an enzyme, produced mainly by parotid gland, which primary function in saliva is to break down high molecular weight carbohydrates to lower molecular weight sugars (i.e., glucose) [(16)]. In addition, amylase seems to play a role in maintaining mucosal immunity [(16,17)]. Hence, such salivary marker (α-Amylase) and flow rate can help to enhance oral defense mechanism.

Due to these detectable issues, this study was conducted to find the correlation between salivary levels of α-Amylase and flow rate with severity of PDs.

MATERIALS AND METHODS

The human sample included 75 males age range from (30-45) years old. Subjects recruited for this study were from the Department of Periodontics at the Teaching Hospital of College of Dentistry, University of Baghdad as well as from blood bank in Baghdad.

From each subject, unstimulated whole saliva sample was harvested, then the amount of saliva in (mL), divided by the time (min) of duration of the collection was recorded as the salivary flow rate, then each sample centrifuged at 3000 rpm for

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10 minutes in the poisons center in Ghazy Alharery Hospital, then the clear supernatant saliva was collected by micropipette into eppendrof tubes and store at -20 ˚C until biochemical analysis of α-amylase.

Full examinations of clinical periodontal parameters were carried out.

1. Assessment of soft deposits by Plaque Index System (PLI) [18].
2. Assessment of Gingival Inflammation by the Gingival Index System (GI) [19].
3. Assessment of Gingival Bleeding on Probing (BOP) [20].
4. Assessment of Probing Pocket Depth (PPD) [20].
5. Assessment of Clinical Attachment Level (CAL) [21].

According to this examination, the subjects generally were divided into three main groups:

1. Chronic periodontitis (CP) Group: Consisted of (45) males had chronic periodontitis. This was defined by the presence of at least four sites with PPD ≥ 4mm and clinical attachment loss of (1-2 mm) or more [22].Patients in this group subdivided into three subgroups according to the severity of clinical attachment loss [21] into:

Mild CP: Consisted of 15 males with clinical attachment loss of 1-2mm.

Moderate CP: Consisted of 15 males with clinical attachment loss of 3-4mm.

Severe CP: Consisted of 15 males with clinical attachment loss of (≥5mm).

2. Gingivitis Group: Consisted of (15) males had gingivitis, this was defined by the presence of signs and symptoms of gingival inflammation and without periodontal pocket or clinical attachment loss.

3. Control Group: Consisted of (15) males with clinically healthy periodontium, this was defined by the absence of any signs and symptoms of gingival inflammation and without periodontal pocket or clinical attachment loss. This group presents a baseline data for the levels of salivary α-amylase.

The inclusion Criteria were apparently systemically healthy subjects or patients and at least 20 teeth present.

The exclusion Criteria were females, smokers, alcohol drinkers, patients undergone periodontal treatment and /or used a course of anti-inflammatory, antimicrobial or other medications in the 3 months before the study and presence of systemic disease ,e.g. Diabetes mellitus, Cardiovascular disease, Rheumatoid arthritis ..etc.

For α-amylase enzyme analysis we used kit manufactured by BioSystems (spain), the kit subjected to modification by a specialist (biochemist) in the Laboratories of the Poisons Center of the specialized surgeries hospital to measure the activity of this enzyme in saliva.

Descriptive statistics in the form of means, standard deviation (S.D.) and inferential statistics in the form of one-way ANOVA test, LSD test and Pearson's correlation coefficient test (r) were used in this study.

In the statistical evaluation, the following levels of significance (Sig.) were used:

Non-significant	NS	$P > 0.05$
Significant	S	$0.05 \geq P > 0.01$
Highly significant	HS	$P \leq 0.01$

We certify that this study involving human subjects is in accordance with the Helsinki declaration of 1975 as revised in 2000 and that it has been approved by the relevant Institutional Ethical Committee.

RESULTS

The mean values of age for control, gingivitis and chronic periodontitis groups were (37.33, 37.47, 37.29) respectively, and these values convergent to each other.

The PLI and GI demonstrated the highest mean values in CP group followed by gingivitis group and lastly the control group, hence the same result was revealed about BOP score1.

In addition to that the severe CP subgroup presented the highest mean values among the CP subgroups followed by moderate CP subgroup and lastly the mild CP subgroup showed the minimum mean values with highly significant statistical differences were observed among the CP subgroups about all clinical periodontal parameters (table-1).

From table -2, comparisons regarding all clinical periodontal parameters revealed highly significant differences between all pairs of CP subgroups with (p-value ≤ 0.001) as well as comparisons of mean values of PLI ,GI,BOP score 1 showed that there were highly significant differences between the Gingivitis group with each one of CP subgroups with p-value ≤ 0.001.

In table -3, the biochemical analysis of salivary α-amylase level revealed that CP group presented the highest mean value (100475.18) followed by gingivitis group (51369.27) and lastly the control group showed the minimum mean value (17403.27). While severe CP subgroup presented the highest mean value

(121266.20) among the CP subgroups followed by moderate CP subgroup (101700.40) then the mild CP subgroup (78458.93). A highly significant statistical difference was observed among the CP subgroups with (p-value≤ 0.000).

On the other hand, physical parameter analysis showed decrease in mean values of FR in gingivitis and CP groups as compared to control group .In addition to that the mild CP subgroup presented the highest mean value (0.85) among the CP subgroups followed by moderate CP subgroup (0.65) and the severe CP subgroup showed the minimum mean value (0.38). A gain a highly significant statistical difference was observed among the CP subgroups with (p-value ≤ 0.001).

Regarding salivary α– amylase and FR, highly significant differences were revealed between all pairs of CP subgroups and between each one of CP subgroups with gingivitis and control groups as well as both groups with each other (table-4).

The results of correlations (table-5) between mean values of PLI and GI for control, gingivitis and CP groups and CP subgroups with the α-amylase levels were highly significant strong positive correlations.

The correlations between mean values of BOP score 1,PPD,CAL of CP group and CP subgroups with the α-amylase were highly significant strong positive , the same result was revealed between mean values of BOP score 1 of gingivitis group with the α-amylase. The correlations between mean values of salivary FR for control, gingivitis and CP groups and CP subgroups with the α-amylase were highly significant strong negative.

From table-6), the correlations between FR with clinical periodontal parameters were almost highly significant strong negative at control, gingivitis and CP groups and CP subgroups.

Table 1: Descriptive statistics of clinical periodontal parameters for groups and subgroups with difference among CP subgroups

Groups and Subgroups	PLI		GI		BOP score 1		PPD		CAL	
	Mean	±S.D.	Mean	±S.D.	Mean ٪	±S.D	Mean	±S.D.	Mean	±S.D.
Control	0.46	0.07	0.35	0.07	-	-	-	-	-	-
Gingivitis	1.47	0.05	1.37	0.03	30.08	1.90	-	-	-	-
CP	2.16	0.34	2.04	0.33	69.17	13.79	2.707	1.885	3.83311	2.026
Mild	1.80	0.05	1.69	0.06	54.75	2.20	0.60	0.06	1.61	0.07
Moderate	2.08	0.09	1.96	0.07	65.52	2.15	2.39	0.11	3.42	0.15
Severe	2.59	0.09	2.47	0.08	87.26	0.82	5.13	0.16	6.46	0.18
F-test among CP subgroups	365.027		494.602		1214.023		5748.496		4425.816	
p-value Sig.	0.000 (HS)		0.000 (HS)		0.000 (HS)		0.000 (HS)		0.000 (HS)	

Table 2: Mean differences of the clinical periodontal parameters between all pairs of CP subgroups and with gingivitis group.

Gingivitis group and CP subgroups		PLI		GI		BOP score 1		PPD		CAL	
		Mean Difference	p-value Sig.	Mean Difference	p-value Sig.	Mean Difference	p-value Sig.	Mean Difference	p-value Sig.	Mean Difference	p-value Sig.
Mild	Moderate	-0.28	0.000 HS	-0.27	0.000 HS	-10.77	0.000 HS	-1.79	0.000 HS	-1.81	0.000 HS
	Severe	-0.79	0.000 HS	-0.78	0.000 HS	-32.51	0.000 HS	-4.53	0.000 HS	-4.85	0.000 HS
Moderate	Severe	-0.51	0.000 HS	-0.51	0.000 HS	-21.74	0.000 HS	-2.74	0.000 HS	-3.04	0.000 HS
Gingivitis	Mild	-0.33	0.000 HS	-0.32	0.000 HS	-24.67	0.000 HS	-	-	-	-
	Moderate	-0.61	0.000 HS	-0.59	0.000 HS	-35.44	0.000 HS	-	-	-	-
	Severe	-1.12	0.000 HS	-1.10	0.000 HS	-57.18	0.000 HS	-	-	-	-

Table 3: Descriptive statistics of salivary α– Amylase concentrations (U/L) and FR (ml/min) for groups and subgroups with difference among CP subgroups

Groups and Subgroups	α – amylase				FR			
	Mean	±S.D.	F-test	p-value Sig.	Mean	±S.D.	F-test	p-value Sig.
Control	17403.27	2227.88			1.34	0.11		
Gingivitis	51369.27	5802.99			1.05	0.04		
CP	100475.18	18175.21			0.63	0.20		
Mild	78458.93	4985.51	381.874	0.000 HS	0.85	0.04	213.713	0.000 HS
Moderate	101700.40	4957.75			0.65	0.04		
Severe	121266.20	2163.88			0.38	0.09		

Table 4: Mean differences of salivary α-Amylase and FR between all pairs of groups and subgroups.

Groups and subgroups		α-amylase		FR	
		Mean Difference	p-value Sig.	Mean Difference	p-value Sig.
Mild	Moderate	-23241.47	0.000 (HS)	0.20	0.000 (HS)
	Severe	-42807.27	0.000 (HS)	0.47	0.000 (HS)
Moderate	Severe	-19565.80	0.000 (HS)	0.27	0.000 (HS)
Control	Gingivitis	-33966.00	0.000 (HS)	0.29	0.000 (HS)
	Mild	-61055.66	0.000 (HS)	0.49	0.000 (HS)
	Moderate	-84297.13	0.000 (HS)	0.69	0.000 (HS)
	Severe	-103862.93	0.000 (HS)	0.96	0.000 (HS)
Gingivitis	Mild	-27089.66	0.000 (HS)	0.20	0.000 (HS)
	Moderate	-50331.13	0.000 (HS)	0.40	0.000 (HS)
	Severe	-69896.93	0.000 (HS)	0.67	0.000 (HS)

Table 5: Correlations between the levels of salivary α-Amylase with the clinical parameters of groups and subgroups

Parameters	Statistical analysis	Control	Gingivitis	CP	Mild	Moderate	Severe
PLI	r	0.955	0.985	0.967	0.951	0.938	0.749
	P-value	0.000	0.000	0.000	0.000	0.000	0.001
GI	r	0.923	0.942	0.966	0.945	0.841	0.853
	P-value	0.000	0.000	0.000	0.000	0.000	0.000
BOP Score 1	r		0.950	0.962	0.765	0.951	0.727
	P-value		0.000	0.000	0.001	0.000	0.002
PPD	r			0.967	0.902	0.882	0.865
	P-value			0.000	0.000	0.000	0.000
CAL	r			0.965	0.883	0.963	0.855
	P-value			0.000	0.000	0.000	0.000
FR	r	-0.945	-0.956	-0.968	-0.887	-0.904	-0.888
	P-value	0.000	0.000	0.000	0.000	0.000	0.000

Table 6: Correlations between the levels of salivary FR with the clinical periodontal parameters of groups and subgroups

Parameters	Statistical analysis	Control	Gingivitis	CP	Mild	Moderate	Severe
PLI	r	-0.934	-0.936	-0.984	-0.911	-0.931	-0.851
	P-value	0.000	0.000	0.000	0.000	0.000	0.000
GI	r	-0.925	-0.891	-0984	-0.952	-0.807	-0.945
	P-value	0.000	0.000	0.000	0.000	0.000	0.000
BOP Score 1	r		-0.901	-0.965	-0.691	-0.908	-0.920
	P-value		0.000	0.000	0.004	0.000	0.000
PPD	r			-0.968	-0.858	-0.851	-0.935
	P-value			0.000	0.000	0.000	0.000
CAL	r			-0.968	-0.745	-0.939	-0.916
	P-value			0.000	0.001	0.000	0.000

DISCUSSION

The mean values of age almost convergent to each other nearly 37 years, this might be due to the selective criteria of patient's age of this study which is (30-45). Since the PDs occur most frequently in patients with older age [23].

In the present study there were highly significant differences between the gingivitis group with each one of CP subgroups were demonstrated concerning PLI, GI, BOP score 1. These were in agreement with the results of other studies [24-27], as well as highly-significant differences among the CP subgroups and between all pairs of subgroups .These findings indicate the effect of plaque accumulation on blood circulation and the actual pathophysiological process that happened more in inflamed tissue and the severity of bleeding with the ease of its provocation depend on the intensity of the inflammation. Where more plaque accumulation with increased number of active sites that coincide with severity of CP, cause proliferation of capillaries and increased formation of capillary loops between rete ridges leads to increase vascular permeability and bleeding tendency [3].

On the other hand, regarding the PPD and CAL highly-significant statistical differences were observed among the CP subgroups and when comparing each two subgroups. Hussein and Mahmood [27] revealed in their study non-significant and highly significant differences among the CP subgroups regarding PPD and CAL respectively. This could be due to increase in the amount of plaque and bacterial invasion that caused destruction of the sulcular & junctional epithelium & surrounding alveolar bone. In addition, the early concepts assumed that after the initial bacterial attack, periodontal tissue destruction continued to be linked to bacterial action [3].

Concerning the α-amylase levels highly significant differences showed among the CP subgroups and in inter subgroups comparisons. On the other hand, comparisons revealed highly significant differences between the control with gingivitis groups, also each of them with each one of CP subgroups. So, increased level of α-amylase with increased severity of PDs.

These results in accordance with other studies which showed an increase in salivary α- amylase levels in patients with gingivitis and chronic periodontitis as compared to control group [15,28-30].

Also in agreement with previous study that was conducted by Kejriwal et al. [31] who showed significant increased levels of salivary α-amylase had been found in patients with gingivitis and chronic periodontitis compared to subjects had clinically healthy periodontium.

While others found highly significant increase in salivary α-amylase level in CP patients as compared to control group [32,33].

Thus, increased levels of α-amylase may be due to the response of salivary glands to inflammatory diseases like gingivitis and periodontitis resulting in increased synthesis and secretion of certain acinar proteins like α-amylase so as to enhance the oral defense mechanism [28,34]. Since, salivary gland secretion is a nerve mediated reflex. Amylase is released by exocytosis from salivary cells in response to sympathetic stimulation [35]. The infectious process of PDs activates the sympathetic system, which in turn leads to the release of some salivary proteins, thereby increasing the protective potential of saliva [15].

Studies showed that α-amylase is a major lipopolysaccharide binding protein of *Aggregatibacter actinomycetemcomitans* and *Propheromonas gingivalis* and interferes with bacterial adherence and biofilm formation [36,37]. Also, it was suggested that the amylase help against streptococcal bacterial adherence, which inhibits further propagation on colonization of bacteria and may help regulate normal bacterial flora in the mouth [17]. Thus, the increase concentration of salivary α-amylase in gingivitis and periodontitis suggests it to be an important defense molecule essential for the innate immunity in the oral cavity [31].

The results showed that FR level decreased in gingivitis group and CP group and subgroups with highly significant differences among the CP subgroups and at inter subgroups comparisons.

At the same time, highly significant differences between the control with gingivitis groups, also between the control and gingivitis groups with each one of CP subgroups were demonstrated.

The decrease in salivary FR of CP group in this study coincide with others [15,33,38].

Some studies [39,40] detected that in CP patients, there was non significant decrease in the FR while others [23] revealed significant decrease in the FR ,although previous study [32] detected highly significant decrease in the FR but non significant increase in the FR as compared to control group was demonstrated[41].

While ,it was found that individuals who have increased salivary inorganic calcium, phosphate, pH, FR with poor oral hygiene could be at a higher risk for developing periodontitis and may have less dental caries and more number of intact teeth [42].

There are multiple causes of salivary hypofunction including inflammation e.g. PDs [33], hydrogen concentration in which the higher concentrations of hydrogen ions (from salivary glands or oral microbiota), the lowest the pH, that can be attributed to decrease in FR [38].

The correlations between mean values of PLI and GI for control, gingivitis and CP groups and CP subgroups with the α-amylase levels were highly significant strong positive correlations .

These are explained by the fact that the microbial biofilm is considered the primary and the major etiological factor responsible for initiation of PD [6].

Thus an increasing in PLI cause increase in the severity of gingival inflammation that leads to increase in the level of α-amylase. This is in agreement with other study [31].

The correlations between mean values of BOP score 1,PPD,CAL of CP group and CP subgroups with the α-amylase were highly significant strong positive , the same result was revealed between mean values of BOP score 1 of gingivitis group with the α-amylase.

These findings can be explained by the fact that increase in severity of inflammation that caused by increase in accumulation of plaque bacteria which demonstrated by increasing in mean values of BOP, PPD and clinical attachment loss [20] accompanied by increasing in salivary α-amylase level [31]. Since it was established that the host's immunoinflammatory response to the initial and persistent bacterial attack unleashes mechanisms that lead to collagen and bone destruction. These mechanisms are related to various cytokines, some produced normally by cells in non-inflamed tissue and others by cells involved in the inflammatory process such as polymorphonuclear leukocytes, monocytes and other cells [20] and this accompanied by increasing in salivary α-amylase level[31].

The correlations between mean values of salivary FR for control, gingivitis and CP groups and CP subgroups with the α-amylase were highly significant strong negative.

Gingivitis and periodontitis induces an increase in the output of total proteins, mucin and α- amylase, as a result of responding of salivary glands to inflammatory diseases thereby increasing the protective potential of saliva and this is accompanied by a decrease in flow rate [15,31].

Sánchez et al. [34] who had been shown that salivary FR had negative correlations with all clinical periodontal parameters in patients with CP. Others [23], demonstrated that the correlations between FR with PLI was significant moderate negative while with CAL significant moderate positive and non-significant weak negative with GI, BOP and PPD.

Thus an increasing in mean values of clinical periodontal parameters due to increase in the severity of inflammation that lead to increase in the level of salivary proteins accompanied by decrease in FR [31,34].

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