Assessment of Alkaline Phosphatase, Salivary Flow Rate and Salivary Potential of Hydrogen in Relation to Severity of Chronic Periodontitis

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ABSTRACT

Background: The cells of periodontium contain many intracellular enzymes like (alkaline phosphatase ALP) that are released outside into the saliva and gingival crevicular fluid (GCF) after destruction of periodontal tissue. The aim of study was to determine the activity of this enzyme in saliva and its relation to the salivary flow rate, PH and clinical periodontal parameters in patients with chronic periodontitis.

Subject, Materials and methods: Sample population consist of 75 individuals ;divided into four groups , the first group (15):control subject, the second group (20):mild chronic periodontitis, the third group(20) moderate chronic periodontitis and the fourth group (20) sever chronic periodontitis, Measurements of plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL), only male were included and saliva was collected from them and subjected to biochemical analysis of the alkaline phosphatase enzyme (ALP), and also measurement of salivary flow rate(FR) and PH.

Results: Statistical analysis of the results revealed the presence of a highly significant difference in the enzymatic activity between healthy and chronic periodontitis subjects (mild, moderate, severe) with positive correlation between the activity of this enzyme and the clinical periodontal parameters, and negative correlation between this enzyme and Salivary flow rate and pH.

Conclusion: From this study it can be concluded that a number of markers show promise as sensitive measures of disease and the effectiveness of therapy. At this time enzymes such as alkaline phosphatase ALP, is good biochemical markers of screening chronic periodontitis. Also ALP can be used as a monitor for healthy individuals and patients with different periodontal diseases. Furthermore, analysis of saliva may offer a cost effective approach to assessment in controlling progression of chronic periodontitis in large populations.

Keyword: Chronic periodontitis, saliva, flow rate, alkaline phosphatase. (J Bagh Coll Dentistry 2016; 28(3):125-131).

INTRODUCTION

Periodontal disease (PD) is one of the common inflammatory diseases within complex etiology and multifactorial in origin. Diagnosis of periodontal disease depended on clinical and radiographic measures of periodontal tissue destruction. These parameters provide measures of past destruction and are of limited use in early diagnosis [1].Genetics and molecular biology in advances stage lead to a better knowledge of the pathways and mechanisms through which microorganism maintain the host immune/inflammatory response [2].

Chronic periodontitis (CP) is very common disease [3]and it is a slowly progressing form of PD, but may have periods of rapid progression [4]. Saliva is an important biological material that got in new diagnostic tests which may contribute in the diagnosis and explaining the pathogenesis of some diseases [5].

Saliva plays an important role in discovery of periodontal disease because it is easily collected and allows analysis of several biological markers such as proteins, enzymes, host cells, hormones, and bacterial products; therefore, no specific laboratory devices are necessary and this approach may be suitable for public health use[6].

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The amount of saliva naturally produced by the salivary glands is called salivary flow rate. Production of saliva is increased by the presence of food or irritating substances, such as vomit, in the oral cavity [7].

Mean daily saliva production for healthy person ranges from 1 to 1.5L [8].The Salivary Flow Index is a parameter allowing stimulated and unstimulated saliva flow to be classified as normal, low, or very low (hyposalivation) [9]. Normal total stimulated Salivary flow rate (FR) ranges from up to 3 ml/ min.

A measure of the acidity or alkalinity of a solution, is defined salivary PH, the normal PH range for saliva is considered to be 5.6 to 7.9, depend on international journal of drug testing's above 7 scale usually indicate alkalinity and when below 7 scale indicate Acidity [10].

Alkaline phosphatase (ALP) is hydrolase enzyme responsible for removing phosphate groups from many of types of molecules, including nucleotides, proteins, and alkaloids. The process of removing phosphate group is called dephosphorlation, ALP is effective in an alkaline environment[11]. This enzyme is an important indicator of bone formation and considered as marker for osteoblast cells. ALP was detected inthe parotid, submandibular and minor salivary glands [12].

MATERIALS AND METHODS

The sample consisted of 75 patients, subjects' collection from the department of Periodontics, at teaching hospital, College of Dentistry, University of Baghdad as well as from blood bank. all subject enrolled voluntarily in the study after a well explanation about the aim and purposes of the study and gave informed consent to participate in the study.

The subjects were divided into four groups according to American Academy of Periodontology 1999[13].

1- Control group I (healthy periodontium): Consisted of fifteen (15) apparently systemically healthy without history of any systemic disease and with healthy periodontium, this was defined by GI scores <0.5 [14].and without periodontal pockets or clinical attachment loss.

2-Study group II (mild chronic periodontitis): Consisted of twenty (20)patients, in which mild clinical attachment loss of (1-2 mm).

3-Study groupIII(moderate chronic periodontitis): Consistedof twenty (20)patients, in which attachment loss up to (3-4 mm).

4- Study groupIV(severe chronic periodontitis): Consisted of twenty (20)patients, in which attachment loss (5 mm) or more.

Exclusion criteria:

1- Patients who have undergone periodontal treatment in the 3 month period prior to the study.
2- A course of anti-inflammatory or antimicrobial therapy during the last (3 months).
3- Smoking or alcohol drinking

Clinical periodontal parameter examination:

1-Assessment of Plaque Index System (PLI):

This was done by using plaque index system which was introduced by Silness [15].

2-Assessment of Gingival Inflammation by the Gingival Index System (GI):

This was assessed using the criteria of the gingival index system that modified by Löe[14].

3- Assessment of Gingival Bleeding on Probing (BOP):

If bleeding occurs within 30 seconds the site was given a positive score **(1)** and a negative score **(0)** for the non –bleeding site[16].

4-Assessment of Probing Pocket Depth (PPD):

This is defined as the distance from gingival margin to the most apical penetration of the periodontal probe inserted into the gingival crevice or pocket without force or pressure

5-Assessment of Clinical Attachment Level (CAL):

It is defined as the distance from the cemento-enamel junction (CEJ) to the location of the inserted probe tip (bottom of gingival crevice or pocket).

Measurement of salivary flow rate and pH:

The volume of unstimulated saliva that collected firstly from each subject at 5 min was recorded; through using graduated syringes, the saliva collected firstly was aspirated from the collection receptacle with a disposable 5 mL sterile syringe avoiding contact with the epithelium. The amount of saliva in mL, divided by the time of duration of the collection was recorded as the mean salivary flow rate. Only the liquid component of the saliva, not the foam, was measured. Where estimation of flow rate (ml/min) was made according to this equation: Flow rate (FR): Volume (ml) / Time (min)) .

Samples containing blood were discarded. The samples were put in a small cooling box after collection to stop bacterial growth. The tube was labeled with the number of the subject corresponding to that written previously on the case sheet. The salivary pH was measured by using the (DP universal test paper) by immersing the strip into the saliva for about 2 seconds, then waited for color changes for 15 seconds and compared to the color chart present on the plastic case of the product.

Biochemical Analysis:

Alkaline Phosphatase (ALP) Enzyme:

For ALP enzyme analysis we used kit manufactured by (BIOMEREIUX ®Sa) which is one of the French leaders of reagents (R) for medical biochemistry, All the kits used for enzymes analysis subjected to modification by a specialist (biochemist) in the laboratories of the poisons center to measure the activity of these enzymes in saliva.

Principle: according to Biomérieux Kit for Alkaline phosphatase enzyme.

Colorimetric determination of ALP activity according to the following reaction:

Phenyl phosphate $\xrightarrow{\text{ALP}}$ phenol + phosphate

The liberated phenol is measured in the presence of 4-aminoantipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction.

Stastistical Analyses

a) **One-way ANOVA test:** to compare the measured variables among the groups.
b) **LSD test:** to test any statistically significant difference between each two groups.
c) **Pearson's correlation coefficient test (r):** to test the relation between ALP and other

clinical periodontal parameter and the measured variables in each group.

RESULTS

The current results revealed that mean values of (PLI, GI, BOP, PPD and CAL)were higher in severe group than other groups, while the mean values of the (FR, pH)were higher in control than other groups and the mean value of (ALP) was higher in sever group than other groups, these results show in table1and 2.

Table 1: Descriptive and inferential statistics of PLI, GI, BOP, PPD and CAL parameter for allstudy groups

Variables	Groups	Descriptive Statistics					Comparison (d.f. =74)	
		N	Mean	S.D.	Min.	Max.	F-test	p-value
PLI	Control	15	0.46	0.07	0.312	0.592	2532.23	0.000 (HS)
	Mild	20	1.79	0.05	1.7	1.875		
	Moderate	20	2.05	0.08	1.89	2.19		
	Severe	20	2.59	0.08	2.44	2.699		
GI	Control	15	0.35	0.06	0.25	0.454	2804.19	0.000 (HS)
	Mild	20	1.70	0.05	1.61	1.77		
	Moderate	20	1.97	0.07	1.873	2.091		
	Severe	20	2.49	0.09	2.3	2.592		
BOP	Mild	20	34.17	4.23	25	37.5	1657.135	0.000 (HS)
	Moderate	20	59.95	2.65	56.25	63		
	Severe	20	87.28	0.80	85.9	88		
PPD	Mild	20	0.60	0.07	0.5	0.75	7633.961	0.000 (HS)
	Moderate	20	2.42	0.13	2.25	2.735		
	Severe	20	5.10	0.13	4.875	5.318		
CAL	Mild	20	1.60	0.09	1.362	1.76	5985.466	0.000 (HS)
	Moderate	20	3.41	0.15	3.11	3.771		
	Severe	20	6.50	0.17	6.096	6.767		

Table 2:Descriptive and inferential statistics of FR, pH and ALP parameter for control and study groups

Variables	Groups	Descriptive Statistics					Comparison (d.f. =74)	
		N	Mean	S.D.	Min.	Max.	F-test	p-value
FR	Control	15	1.38	0.11	1.2	1.5	429.54	0.000 (HS)
	Mild	20	0.87	0.04	0.8	0.9		
	Moderate	20	0.67	0.04	0.6	0.7		
	Severe	20	0.38	0.12	0.2	0.5		
pH	Control	15	7	0	7	7	318.996	0.000 (HS)
	Mild	20	5.6	0.5	5	6		
	Moderate	20	4.30	0.47	4	5		
	Severe	20	2.65	0.49	2	3		
ALP	Control	15	23.57	1.28	21.22	25.05	695.31	0.000 (HS)
	Mild	20	74.40	9.50	60.25	89.38		
	Moderate	20	98.32	5.32	91.20	107.04		
	Severe	20	119.23	5.76	109.41	131.62		

Inter-groups comparisons by Least significant difference (LSD) test for testing equality of variances illustrated in the table (3, 4), The statistical analysis using the LSDtest to compare mean values of (PI, GI, BOP, PPD, CAL),(ALP, FR, PH) between study groups, revealed highly significant differences at $p<0.01$.

Table 3: Inter-groups comparisons of mean value of PLI, GI, BOP, PPD and CAL parameter between study groups

Variables	Groups		Mean Difference	p-value
PL	Mild	Moderate	-0.26	0.000 (HS)
		Severe	-0.80	0.000 (HS)
	Moderate	Severe	-0.54	0.000 (HS)
GI	Mild	Moderate	-0.27	0.000 (HS)
		Severe	-0.79	0.000 (HS)
	Moderate	Severe	-0.51	0.000 (HS)
BOP	Mild	Moderate	-25.79	0.000 (HS)
		Severe	-53.12	0.000 (HS)
	Moderate	Severe	-27.33	0.000 (HS)
PPD	Mild	Moderate	-1.82	0.000 (HS)
		Severe	-4.50	0.000 (HS)
	Moderate	Severe	-2.68	0.000 (HS)
CAL	Mild	Moderate	-1.81	0.000 (HS)
		Severe	-4.90	0.000 (HS)
	Moderate	Severe	-3.09	0.000 (HS)

Table 4: Inter-groups comparisons of mean value of pH, FR, and ALP parameter between all four groups

Variables	Groups		Mean Difference	p-value
pH	Control	Mild	1.4	0.000 (HS)
		Moderate	2.7	0.000 (HS)
		Severe	4.35	0.000 (HS)
	Mild	Moderate	1.3	0.000 (HS)
		Severe	2.95	0.000 (HS)
	Moderate	Severe	1.65	0.000 (HS)
FR	Control	Mild	0.51	0.000 (HS)
		Moderate	0.71	0.000 (HS)
		Severe	1	0.000 (HS)
	Mild	Moderate	0.20	0.000 (HS)
		Severe	0.49	0.000 (HS)
	Moderate	Severe	0.29	0.000 (HS)
ALP	Control	Mild	-50.83	0.000 (HS)
		Moderate	-74.75	0.000 (HS)
		Severe	-95.66	0.000 (HS)
	Mild	Moderate	-23.92	0.000 (HS)
		Severe	-44.83	0.000 (HS)
	Moderate	Severe	-20.90	0.000 (HS)

Regarding the correlation coefficient between level of ALP enzyme and PLI, GI Parameters for control group strong highly-significant positive correlation was found, while with Salivary FR, it showed a strong highly -significant negative correlation, these results were demonstrated in table (5).

Table 5: Pearson's Correlation Coefficient (r) between ALP and PL, GI, FR and PH of the control group

Variables		PI	GI	FR	PH
ALP	r	0.903	0.930	-0.970	-
	p-value	0.000	0.000	0.000	-

The results of correlation coefficient between PLI, GI, BOP, PPD, CAL Parameters and level of the ALP enzyme for mild group, revealed a strong highly-significant positive correlation.The results between FR, PH Parameters and ALP, revealed a strong highly-significant negative correlation. These results were demonstrated in table (6).

Table 6:Pearson's Correlation Coefficient (r) between Clinical Periodontal Parameters (PLI, GI, BOP, PPD, CAL) and biochemical parameters(ALP) with FRandPHof themild group.

Variables		PI	GI	BOP	PPD	CAL	FR	pH
ALP	r	0.682	0.694	0.774	0.704	0.708	-0.814	-0.779
	p-value	0.001	0.001	0.000	0.001	0.001	0.000	0.000

Regarding the correlation coefficient between PLI, GI, BOP, PPD, CAL Parameters and level of the ALP for moderate group, the results revealed a strong highly-significant positive correlation. The results of correlation coefficient between FR, PH Parameter and ALP, revealed a strong highly-significant negative correlation, these results were demonstrated in table (7).

Table 7: Pearson's Correlation Coefficient (r) between Clinical Periodontal Parameters (PLI, GI, BOP, PPD, CAL) and biochemical parameters (ALP) with salivary flow rate of the moderate group.

Variables		PI	GI	BOP	PPD	CAL	FR	pH
ALP	r	0.956	0.892	0.981	0.951	0.928	-0.846	-0.784
	p-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

The results of correlation coefficient between PLI, GI, BOP, PPD, CAL Parameters and level of the ALP for severe group, revealed a strong highly-significant positive correlation. The result between FR, PH Parameters and ALP, showed a strong highly-significant negative correlation, these results were demonstrated in table (8).

Table 8: Pearson's Correlation Coefficient (r) between Clinical Periodontal Parameters (PLI, GI, BOP, PPD, CAL) and biochemical parameters (ALP) with salivary flow rate of thesevere group.

Variables		PI	GI	BOP	PPD	CAL	FR	pH
ALP	r	0.965	0.937	0.780	0.969	0.957	-0.838	-0.701
	p-value	0.000	0.000	0.000	0.000	0.000	0.000	0.001

DISCUSSION

The findings of the study that a highly significant difference in PLI, GI,BOP, PPD, CAL between the study and control groups, these findings were due to increase in the bacterial invasion and the amount of plaque that caused destruction of the sulcular and junctional epithelium and surrounding alveolar bone.

This findings revealed that the level of Alkaline Phosphatase ALP in the study group higher than control groups these results were in agreement with Nakamura[17].Also accordance with many studies [18,19].

The explanation for this difference in the enzyme activity between the two groups may be due to the fact that ALP is present at or near the cell membrane of alveolar bone osteoblasts and fibroblasts of the periodontal ligaments [20-22].During the active stages of periodontitis, there will be destruction of alveolar bone osteoblasts and fibroblasts and their cell membrane will be ruptured releasing their intracellular contents outside. So ALP will be released into saliva and GCF and the level of ALP will increase in saliva [6,23,24].

In the present study there was statistically high-significant differences in unstimulated salivary flow rate among either groups[25] indicate that Periodontitis induces an increase in the output of proteins, thereby enhancing the protective potential of saliva, but this is accompanied by a decrease in flow rate.

But disagree with Fiyaz[26] who found that individuals who have increased salivary inorganic calcium, phosphate, pH, FR and maintain poor oral hygiene could be at ahigher risk for developing periodontitis and may have less dental caries and more number of intact teeth.

The findings of the study showed that there was a highly-significant difference in term of salivary PH, between study and control groups, Baliga [27] observed that there is a correlation between pH of saliva and periodontal diseases when compared with healthy groups. Salivary pH in patients with chronic generalized periodontitis is more acidic than the control group, increase acidity (pH below 7) as the increasing severity of periodontal condition.

This results revealed that a strong highly-significant positive correlation between ALP and PL, GI index in control group, also between ALP

and PL, GI, BOP, PPD, CAL indices in mild, moderate and severe groups respectively. The explanation for this result is that ALP produced by many cells such as PMNLs during inflammation, from osteoblasts [28] and PDL fibroblasts [29]during bone formation and periodontal regeneration respectively, the usual immunological response to dental plaque pathogens and alveolar bone destruction makes the ALP concentration correlated positively with clinical periodontal parameters.

This result agreed with other studies, the findings of Todorovic [30] showed a high coefficient of correlation between the values of GI and the activities of these enzymes. Number of previous studies disagreed with our results, these findings in Herasaki [31]. As they found that there was no correlation between ALP and PLI, GI Parameter. The findings of the study were faced by Ray [32]. Who stated that there was no correlation between the enzymatic activity of ALP and BOP.

This result stated that there was correlation between the activities of these enzymes and PPD and CAL, These findings were not supported by Herasaki [31].On the other side, the study of Ray [32] concluded that ALP had a significant correlation with PPD and CAL.

Also our results revealed that a strong highly-significant negative correlation between ALP with FR in control, mild, moderate, severe groups [25] indicate that periodontitis induces an increase in the output of proteins, thereby enhancing the protective potential of saliva, but this is accompanied by a decrease in flow rate (increasing severity the inflammation lead to highly protein concentration lead to decrease salivary flow rate).

Also this result revealed that a strong highly-significant negative correlation between ALP with pH in mild, moderate, severe groups [27] observed that there is a correlation between PH of saliva and periodontal diseases when compared with healthy groups. Salivary pH in patients with chronic generalized periodontitis is more acidic than the control group, increase acidity (pH below 7) as the increasing severity periodontal condition.

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