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Salivary Alkaline Phosphatase and Periodontal Disease

Dr. Nadia A. Al- Rawi, B.D.S., M.Sc.
Dr. Jinan Mohammed Rashad, B.D.S., M.Sc.
Dr. Baidaa Hussain, B.D.S., M.Sc.

Abstract

Saliva is an easily collected fluid that contains locally and systemically derived markers of periodontal disease. The purpose of this study was to compare the level of salivary alkaline phosphatase activity in healthy subjects and patients with periodontal disease (gingivitis and periodontitis), and to evaluate the relationship of this enzyme with different clinical parameters in all patients included in the study**.**

The study included 65 patients referred to Department of Oral Diagnosis, College of Dentistry, University of Baghdad, they were divided into three groups, 20 healthy subjects, 20 subjects with gingivitis and 25 one with periodontitis. The clinical parameters including plaque index (PI), gingival index (GI), probing pocket depth (PPD) and clinical attachment loss (CAL) were recorded and salivary samples collected for analysis of alkaline phosphatase (ALP) levels.

A highly significant increase in mean values of clinical parameters was found in patients with periodontal disease (gingivitis and periodontitis) compared to healthy subjects, also a highly significant increase in the mean values of alkaline phosphatase activity was illustrated as the severity of periodontal disease increased. ALP level was closely related to the gingival index, probing depth and attachment loss in patients with periodontal disease, while no relation was shown to plaque index.

Salivary alkaline phosphatase could be used as a useful marker for monitoring periodontal disease.

Key words: alkaline phosphatase, plaque index, probing depth.

Introduction

Periodontal disease is one of the common inflammatory diseases with complex etiology and multifactorial in origin. The disease state ranges from gingivitis to periodontitis. Gingivitis is characterized by inflammation of the gingiva caused by plaque deposits, with possible bleeding when brushed or probed, while periodontitis is an inflammatory disease affects the supporting tissues of the teeth, leading to progressive destruction of connective tissue attachment and alveolar bone [(1)]. Diagnosis of periodontal disease has been primarily based upon clinical and radiographic measures of periodontal tissue destruction. These parameters provide a measure of past destruction and are of limited use in early diagnosis [(2)]. Traditional periodontal diagnostic procedures are not precise and only allow retrospective assessment of attachment loss. Considerable

improvement and advances have been achieved in improving the accuracy of traditional clinical diagnostic procedures and in developing new diagnostic techniques. Continuing efforts have been directed to improve effectiveness of these diagnostic strategies, which in addition to diagnosis can be extremely useful in evaluating treatment outcomes and patient monitoring for disease recurrence [3-5]. Saliva has been used as a diagnostic fluid in medicine and dentistry [6, 7]. Components of saliva proposed as disease markers, among the important saliva components, there are various enzymes. Intracellular enzymes are increasingly released from the damaged cells of periodontal tissues into the gingival crevicular fluid (GCF) and saliva. Several enzymes that are evaluated for the early diagnosis of periodontal disease are aspartate and alanine aminotransferase (AST, ALT), lactate dehydrogenase (LDH), creatine kinase (CK), alkaline and acid phosphatase (ALP, ACP), and gamma glutamil transferase (GGT) [8-10]. The enzyme ALP plays a role in bone metabolism. It is a membrane-bound glycoprotein produced by many cells, such as polymorphonuclear leukocytes, osteoblasts, macrophages, and fibroblasts within the area of periodontium and gingival crevice [11]. Gao et al (1999) found that ALP activity was highest in osteoblasts, moderate in periodontal ligament PDL fibroblasts, and lowest in gingival fibroblasts. No activity was detected in cementoblasts [12]. In the periodontium, ALP is very important enzyme as it is a part of normal turnover of periodontal ligament, root cementum and maintenance of these tissues, and bone homeostasis. Some forms of enzyme are also produced by plaque bacteria [13]. Some studies have shown a remarkably increased activity of ALP in the acute phase of periodontal disease, but after the periodontal therapy, the activity of these enzymes restored to the value as found with the healthy persons [14]. ALP level has often been measured in GCF to examine the relationship between periodontal conditions and disease activity [11, 15, 16]. Plagnat et al (2002) studied ALP in GCF from implants with and without peri-implantitis and suggested that ALP could be a promising marker of bone loss around dental implants [17]. Gilbert et al (2003) studied ALP activity in serum from patients with chronic periodontitis and showed a relationship between loss of attachment in periodontal disease and ALP activity in serum [18]. Perinetti et al (2002) suggested that ALP amount in GCF reflects the biologic activity in the periodontium during orthodontic movement [19]. Todorovic et al (2006) examined the activity of CK, LDH, AST, ALT, GGT, ALP and ACP in saliva from patients with periodontal disease before and after periodontal treatment. They concluded that the activities of CK, LDH, AST, ALT, GGT, ALP and ACP enzymes were significantly increased in the saliva of patients with periodontal disease when compared to healthy subjects [20]. Daltaban et al (2006) found no significant differences in the concentrations of ALP between periodontitis and control groups [21] however, most of the above studies are related to levels of ALP in GCF of patients with periodontal disease, and few studies have evaluated salivary ALP levels in patients with periodontal disease.

The aim of the present study was to evaluate the level of alkaline phosphatase activity in salivary sample from healthy subjects and patients with gingivitis and periodontitis.

Materials and Method

A total of 65 patients aged 35-50 (mean age 41.44 ± 4.19 years) of both sexes were included in this study. All cases were referred to the Department of Oral Diagnosis, College of Dentistry, University of Baghdad. Patients were divided into three groups, twenty-five patients with periodontitis, 20 with gingivitis and 20 healthy subjects as a control group. All subjects had a good general health with no history of systemic diseases. Pregnant and lactating females were excluded, post-menopausal females or others on estrogen therapy were also excluded. Selection criteria of gingivitis patients were the presence of varying degrees of gingival inflammation with bleeding on probing. The periodontal healthy individuals were selected on the basis of no radiographic evidence of alveolar bone and attachment loss, with probing pocket depths (PPD) lesser than 3 mm and no significant bleeding on probing. Periodontitis was diagnosed when depth of periodontal pocket ≥ 3mm. The clinical evaluation of patients was based on the following parameters; the plaque index (PI) to measure the amount of plaque [22], gingival index (GI) to measure the gingival inflammation [23], probing pocket depths (PPD) and attachment level (AL). The probing pocket depth (PPD) and clinical attachment loss (CAL) measures were obtained at six sites around each tooth (Mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid lingual, and disto-lingual, using a WHO periodontal probe. The probe was directed parallel to the long axis of the tooth. PD was measured the distance from the gingival margin to the bottom of the pocket for each tooth. The measurements of the CAL were recorded in mm by reading of the distance form cemento-enamel junction to the base of the probeable crevice or in other cases indirectly by subtracting the distance from the gingival margin to the cemento-enamel junction from the pocket depth. The whole saliva was collected by unstimulated passive drool into labeled plastic polyethylene tubes, after saliva collection, the samples were placed on ice immediately, then the plastic tubes were frozen – stored until biochemical analysis which was done within one month. The analyses of samples were done in Teaching Laboratories-Medical City Hospital – Baghdad. ALP was determined by Colorimetric method using kit supplied by Bio-Mariner Company – Germany. The values were expresses as unit/liter (U/L). The applied statistical analyses were the following: mean value, standard deviation, correlation co-efficient (Pearson), and ANOVA test using SPSS version 14.

Results

(Mean and standard deviation) of clinical parameters for healthy, gingivitis and periodontitis subjects are presented in table (1). There were increasing in the values of all clinical parameters in gingivitis and periodontitis compared to the healthy subjects with a highly significant difference (Table 2, 3, 4, and 5). Periodontitis showed the highest values, gingivitis was come next, while the lowest values were recognized for the healthy subjects.

Table (6) shows the mean values and standard deviation of alkaline phosphatase activity for healthy, gingivitis and periodontitis patients. Elevated salivary level of alkaline phosphatase activity was observed in the periodontitis and gingivitis patients compared to the healthy one with highly significant differences among the three groups (Table 7, $P<0.001$).

Table (8) demonstrates the correlation of salivary alkaline phosphatase to different clinical parameters, alkaline phosphatase did not show any correlation to PlI in all three groups of subjects. While a good significant correlation of alkaline phosphatase activity was determined to gingival index, probing pocket depth and clinical loss of attachment in patients with gingivitis and periodontitis, and a weak correlation was revealed between ALP activity and gingival index, pocket depth and clinical loss of attachment in healthy subjects. It seems that alkaline phosphatase activity was increased with increasing severity of periodontal disease.

Discussion

Periodontal disease is one of the commonest diseases that may progress to the resorption of alveolar bone, which leads to progressive bone destruction and eventually tooth loss will occur. As a consequence of resorption, breakdown of products or enzymes are released into periodontal tissues, migrating toward the gingival sulcus and gathering from the surrounding site in whole saliva, where several of them have been identified [24]. Among the several host enzymes proposed as diagnostic indicators of periodontal status is ALP which was one of the first to be identified [25]. This study was conducted to determine the level of this enzyme in healthy subjects compared to patients with gingivitis and periodontitis.

Previous studies mainly investigated the activities of this enzyme in gingival crevicular fluid, which is in a much closer contact with periodontal tissues and, due to this, it surely much better reflects the occurrences in them [11, 15, 19, 21]. However, the problem with the gingival crevicular fluid is in that the technique of its collection is rather complicated. Contrary to the gingival crevicular fluid, there is plenty of saliva, the procedure of its sampling is much easier and more bearable for the patient and, however, the same enzymes as those in the gingival crevicular fluid can be detected [20]. For all of the above reasons saliva samples were taken for analysis of ALP activity instead of GCF samples.

In the present study, plaque index, gingival index, probing pocket depth and clinical loss of attachment were measured in the three groups. A highly significant difference was found among healthy, gingivitis and periodontitis patients. There is no doubt that bacterial plaque is a major cause of the initiation and maintenance of gingival inflammation as well as pocket formation and attachment loss.

In this study, the increase in the activity of alkaline phosphatase was shown in patients with gingivitis and periodontitis compared to healthy subjects, the difference was highly significant among the three groups. The increased activity of ALP in periodontal disease may be due to an increase in the inflammation and bone turnover rate. This is probably a consequence of pathological processes in periodontal tissues as ALP is produced by PMNs, osteoblasts, macrophages, fibroblasts and plaque bacteria within periodontal tissues or periodontal pocket [11, 25].

This study showed a non significant correlation between the activity of salivary ALP and plaque index in all of the three groups of subjects while a significant positive correlation was found between ALP in saliva and the values of gingival index, pocket depth and clinical loss of attachment in patients with gingivitis and periodontitis. Early investigations of ALP and periodontal disease in an

experimental gingivitis model showed a significant correlation between ALP and pocket depth and between ALP and inflammation [25]. Nakamura and Slots studied a total of 76 enzyme activities in mixed whole saliva and noted higher enzyme activity in individuals with periodontal disease than non-diseased individuals [26]. Supporting these results are the findings of Todorovic et al that revealed an increased activity of salivary ALP is seen in patients with periodontal disease in relation to a non-disease control group. Diseased group further showed a positive correlation between the salivary enzyme activity and gingival index values [20].

On the basis of results of this study salivary alkaline phosphatase activity can be considered as a biochemical marker of the functional condition of periodontal tissues what provides new opportunities in making diagnosis and prognosis of periodontal disease. However, more studies are necessary to evaluate which specific clinical, microbiological and histological characteristics of periodontal disease are associated with elevated level of alkaline phosphatase in saliva.

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Table (1): Mean, and Standard Deviation of Clinical Parameters for Healthy Subjects, and Patients with Gingivitis and Periodontitis.

Variable	Healthy (n= 20)		Gingivitis (n= 20)		Periodontitis (n= 25)	
	Mean	SD	Mean	SD	Mean	SD
Pl I	0.43	0.30	0.84	0.18	1.53	0.34
GI	0.41	0.15	0.90	0.09	1.66	0.24
Probing depth (mm)	1.95	0.43	2.45	0.51	3.78	0.66
Clinical loss of attachment (mm)	0.91	0.32	2.05	0.58	3.63	0.61

Table (2): ANOVA Test among Variables of Plaque Index for Healthy, Gingivitis and Periodontitis.

	Sum of square	d.f.	Mean square	F- value	P value
Among groups	14.087	2	7.044	86.761**	0.000
Within groups	5.033	62	0.081		
Total	19.121	64			

** Highly Significant P<0.001.

Table (3): ANOVA Test among Variables of Gingival Index for Healthy, Gingivitis and Periodontitis.

	Sum of square	d.f.	Mean square	F- value	P value
Among groups	17.934	2	8.967	291.896**	0.000
Within groups	1.905	62	0.031		
Total	19.838	64			

** Highly Significant P<0.001.

Table (4): ANOVA Test among Variables of Pocket Depth for Healthy, Gingivitis and Periodontitis.

	Sum of square	d.f.	Mean square	F- value	P value
Among groups	40.978	2	20.489	66.931**	0.000
Within groups	18.980	62	0.306		
Total	59.958	64			

** Highly Significant P<0.001.

Table (5): ANOVA Test among Variables of Clinical Loss of Attachment for Healthy, Gingivitis and Periodontitis.

	Sum of square	d.f.	Mean square	F- value	P value
Among groups	84.233	2	42.117	152.576**	0.000
Within groups	17.114	62	0.276		
Total	101.348	64			

** Highly Significant P<0.001.

Table (6): Mean (U/L) and Standard Deviation of Salivary Alkaline Phosphatase Activity of Healthy, Gingivitis and Periodontitis Patients.

	Sum of square	d.f.	Mean square	F- value	P value
Among groups	4183.386	2	2091.693	154.670**	0.000
Within groups	838.460	62	13.524		
Total	5021.846	64			

(7): ANOVA Test among Variables of Alkaline Phosphatase for Healthy, gingivitis and periodontitis subjects.

Variable	**Healthy**	**Gingivitis**	**Periodontitis**			
Alkaline phosphatase enzyme (ALP)	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
	8.35	2.25	14.25	3.55	24.84	5.19

** Highly Significant P<0.001.

Table (8): Pearson Correlation Coefficient between Clinical Parameters and ALP in Healthy, Gingivitis and Periodontitis Patients.

Parameter	**ALP**					
	Healthy		**Gingivitis**		**Periodontitis**	
	r	**p-value**	**r**	**p-value**	**r**	**p-value**
PlI	-0.006	0.98	0.07	0.77	-0.08	0.70
GI	0.36	0.12	0.54*	0.02	0.58*	0.002
Probing pocket depth	0.30	0.20	0.53*	0.02	0.58*	0.003
Clinical loss of attachment	0.42	0.06	0.53*	0.02	0.60*	0.002

*p < 0.05.

r, Pearson correlation coefficient.