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Original Research Article.

Salivary Alkaline Phosphatase, Calcium and Phosphorous as Biochemical Markers in Chronic Periodontal Disease

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ABSTRACT

Background: Alkaline phosphatase (ALP), Calcium and Phosphorous are biomarkers used in inflammatory diseases of the hard and soft tissue and they can serve as a useful tool to measure the efficacy of the non-surgical periodontal therapy.

Objectives: this study aimed to determine the salivary alkaline phosphatase activity, salivary calcium and phosphorous in chronic periodontitis before and after the oral hygiene improvement, scaling, and root planning.

Materials and methods: Forty subjects were selected from UQUDENT teaching hospital and classified into 2 groups: Group 1 (20 adult healthy subjects), Group 2 (20 adult chronic periodontitis patients). Saliva was collected from each case for determination of alkaline phosphatase activity, salivary calcium and phosphorous spectrophotometrically using commercial kits before and after patients receive periodontal treatment.

Results: The salivary calcium and phosphorous levels were higher but non-significant in healthy subject than chronic periodontitis patients before treatment. The calcium level is significantly increased after treatment, while phosphorous level is non-significantly decreased after treatment. Alkaline phosphatase activity is significantly decreased in chronic Periodontitis patients before the treatment compared with the healthy subjects and is significantly decreased in chronic periodontitis after treatment than before treatment.

Conclusion: It could be concluded that increase in the salivary alkaline phosphatase level and decreased salivary calcium level in chronic periodontitis may be considered as biochemical markers for the detection and progression of periodontal disease.

Key words: Chronic Periodontitis, Saliva, Alkaline phosphatase, salivary calcium, Salivary phosphorous

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INTRODUCTION

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with increased probing depth formation, recession, or both. The most common form of periodontitis is the chronic type which is associated with accumulation of plaque and calculus. It has a slow rate of disease progression with a period of increase the destruction activity that's being due to local, systemic, or environmental factors that may influence the normal host-bacteria interaction. Chronic periodontitis has different degrees of severity which includes: mild: Clinical attachment loss (CAL) = 1 - 2 mm; moderate: CAL= 3 - 4mm; severe: CAL ≥ 5m.[1] Diagnosis of chronic periodontitis depends on taking the history from the patient, probing depth (PD), bleeding on probing (BOP), and gingival criteria evaluation as Gingival Index (GI). Also, biomarkers have an important role in the diagnosis of periodontal disease.[2] Saliva contains many locally and systemically derived markers to detect the presence of periodontal disease.[3] The association between different salivary biomarkers and the clinical manifestation of periodontal disease has been evaluated from many aspects which include: inflammation, collagen degradation and bone turnover.[4] The enzymes that indicate tissue degradation are aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP) and acid phosphatase (ACP).[5] However, if a periodontal tissue is destructed, or cells become damaged, these intracellular enzymes are increased in the gingival crevicular fluid (GCF) and saliva, where their activity can be measured.[6]Several studies have correlated the levels of these enzyme activity in GCF and saliva

with the severity of periodontal disease.[4,7,8] Alkaline phosphatase (ALP) (orthophosphoric-monoester phosphohydrolase) is a membrane-bound glycoprotein produced by many cells such as polymorphonuclear leukocytes, osteoblasts, macrophages, and fibroblasts in the periodontium and gingival crevice. It is released from neutrophils during inflammation, osteoblasts during bone formation and periodontal ligament fibroblasts during periodontal regeneration. So, ALP is considered as an important msrker of osteoblastic activity. It has a dual involvement in both processes of periodontal inflammation, healing and regeneration. It is stored in specific granules and secretory vesicles in neutrophils and is mainly released during their migration to the site of infection.[9] ALP enzyme is always detected in major and minor salivary glands, epithelial cells, leukocytes and dental plaque bacteria.[8]Saliva contains the most important electrolytes of the body fluid (calcium, phosphorous and other minerals).[10] They are exerting a major influence on the initiation, maturation and metabolism of dental plaque.[11] The concentration of salivary calcium and phosphorous are important for periodontal heath, as increased level of salivary calcium or phosphorous is related to rapidly mineralized plaque, associated with poor oral hygiene.[12,13] Therefore, salivary biomarkers like salivary calcium, phosphorous, alkaline phosphatase and pH can be considered for evaluating the diagnosis and prognosis of gingivitis or periodontitis.[14] The aim of this study was to evaluate salivary alkaline phosphatase activity, salivary calcium and phosphorous in chronic periodontitis patients before and after the oral hygiene improvement by reported instruction, scaling, and root planning.

MATERIALS AND METHODS

Subjects

Forty subjects selected from those attending the UQUDENT teaching hospital, college of dentistry at Umm-Al-Qura University in Makkah. They were divided into two groups: Group 1 (20 healthy control subjects). Group 2 (20 subjects with chronic periodontitis). Both groups were matched regarding age and gender.

Inclusion and exclusion criteria

All subjects were with good general health and no history of systemic diseases and have not less than 15 teeth in the mouth. Pregnant and lactating females, post- menopausal females or those on steroid therapy were excluded. Smokers, alcoholics and patient who had taken antibiotics in the past 6 months or those who were subjected to periodontal treatment in the past 6 months were also excluded.

Clinical examination and treatment

Each subject signed an informed consent and received a complete clinical periodontal examination which includes BOP, PD and CAL measurement and gingival criteria evaluation according to Leo and Silence[15] which is recording both soft debris and mineralized deposits [0 = no plaque detected, 1 = looks clean but material can be removed from gingival 1/3 with probe, 2 = visible plaque, 3 = Tooth covered with abundant plaque]. Twenty adult patients who have chronic periodontitis received a conventional periodontal treatment consisting of oral hygiene instructions, scaling, and root planning.

Saliva collection and analysis

Each subject was asked to rinse his/her mouth with water to remove any debris or exfoliated cells. The non- stimulated saliva was collected in a sterile test tube before receiving any periodontal treatment and immediately stored in the refrigerator (2–8 °C) and then were centrifuged at 10,000 rpm for 10 min and the supernatants were stored at (-20 °C) until the time of assay.

Assay of ALP activity, Ca and P in saliva

The level of salivary ALP activity, Ca and P levels in the were determined spectrophotometrically using commercial kit according to manufacture instructions (Human Diagnostics, Wiesbaden, Germany)Saliva was collected and analyzed again 4-6 weeks after the patients received the conventional periodontal treatment

Ethical Approval

Ethical approval for this research was obtained from UQUDENT ethical committee.

Statistical Analysis

Data were tabulated and analyzed by using Statistical Package for Social Sciences (SPSS) version 24.0. Unpaired T-test used to test for statistically significant differences between control group and chronic periodontitis patients group before receiving periodontal treatment while, paired T-test was used to compare the differences between diseased groups before and after receiving the treatment.

Table1: Salivary ALP activity, Calcium and Phosphorous in studied groups; includes values before and after treatment of chronic periodontitis.

	Control	Chronic periodontitis			
		Pre-Treatment	*P*- value	Post- Treatment	*P*-value
Calcium	2.22±1.11	1.93±0.62	0.77	2.75±1.25	0.04*
Phosphorous	10.52±5.25	9.40±4.39	0.650	8.20±4.63	0.346
Alkaline Phosphatase	6.19±5.86	27.6±33.69	0.02*	16.02±18.45	0.346

*Statistically significant (P < 0.05). Values are showed as Mean ± SD.

RESULTS

The results of the present study showed that the level of salivary calcium in Group1, Group 2 pre-treatment and Group 2 post-treatment were 2.22±1.11, 1.93±0.62, 2.75 ±1.25 respectively. There was no statistically significant difference between Group 1 and Group 2 (P>0.05), while there was significant difference in the mean values between Group 2 pre-treatment and Group 2 post-treatment (P < 0.05) (Table 1).The mean value of salivary phosphorous is 10.52±5.25 for Group 1, 9.40±4.39 for Group 2 pre-treatment 8.20±4.63 for Group 2 post-treatment , there was no significant difference between all three groups (P > 0.05) (Table 1).The mean values of salivary of Alkaline phosphatase in Group 1, Group 2 pre-treatment and Group 2 post- treatment were 6.19±5.86, 1, 27.6±33.69 and

16.02±18.45 respectively. Significant difference was observed between Group 1 and Group 2 also between pre-treatment and Group 2 post-treatment (P <0.05) (Table 1). The salivary calcium in healthy subject was slightly higher but not significant than the chronic periodontitis patients before the treatment, while the calcium level is increased significantly after non-surgical treatment.The salivary Phosphorous level in healthy subject was slightly higher than chronic periodontitis before the treatment, while its level in the chronic periodontitis after treatment is slightly less than the level of it before the treatment.ALP activity in chronic periodontitis before the treatment was significantly higher than the healthy subjects and the level of ALP is then decreased after non-surgical treatment.The number of the diseased samples is reduced due to the attrition occur after treatment.

DISCUSSION

Saliva plays an important role in maintenance of oral health. Changes in composition and output of saliva may have detrimental effects on oral health.[16] Recent advances in diagnosis of oral and periodontal diseases are moving towards use of various biomarkers, which aid to identify and quantify the periodontal risk. In traditional periodontics, the clinical criteria are mostly insufficient for detecting the sites with active disease, monitoring the treatment response and measuring the degree of susceptibility for future disease progression. The Presence of specific biomarkers associated with the pathogenesis of periodontal diseases makes saliva a noninvasive, valuable source of clinically relevant information about oral health and disease.[17]The results of the present study revealed that the salivary calcium in healthy subjects is higher but nonsignificant than the chronic periodontitis before treatment, while it is significantly increased after non-surgical treatment. These results are consistent with previous studies which concluded that, higher salivary calcium level was related to good dental health and there was no relation to periodontal bone destruction.[18, 19] Suresh *et al.*[20] concluded that the salivary calcium level in healthy group was more than the level in periodontitis patients group. However, RufiMurad Patel *et al.*[14] demonstrated that the subjects with periodontitis have significantly higher levels of salivary calcium than gingivitis and healthy group. In this study salivary phosphorus levels revealed no significant difference between all three groups ($P > 0.05$). Fiyaz*et al.*[21] reported that there was a positive correlation between high salivary phosphorous content and periodontitis. In contrary, Kolte A *et al.*[22] showed that smokers with periodontitis exhibited statistically significant reduced levels of total proteins, calcium, phosphorus and magnesium compared to nonsmokers with periodontitis. In the present study we could not demonstrate the effect of smoking as all subjects were non-smokers. The fluctuations in dietary calcium intake and general calcium turnover may reflect the level of salivary calcium. In the present study we could not demonstrate the effect of diet. Literatures suggested that, the affinity of salivary calcium to be readily taken up by dental plaque plays an important role in onset of periodontal diseases.[18] in the present study, salivary ALP activity was found to be significantly higher in chronic periodontitis before treatment than the healthy subjects and its level is significantly decreased after non-surgical treatment. Salivary ALP activity was significantly raised in chronic periodontitis subjects as compared to healthy subjects. Previous studies are in agreement with this study.[7, 8, 23] the rise in the ALP activity may be due to tissue alteration as a result of host parasite reaction. During progression of the disease, enzymes are released from dead and dying cells of the periodontium, polymorphonuclear leukocytes, inflammatory, epithelial, and connective tissue cells of the affected sites.[24,25] These results are in accordance with a previous study which concluded that salivary ALP can be used as a biomarker as it reflects the inflammation and destruction of periodontal tissues.[23] It can be concluded that the salivary calcium, phosphorous and alkaline phosphatase levels may be used as potential biochemical markers for the detection and progression of periodontal disease. More research may be required to study the mechanism of action of these salivary biomarkers in periodontal disease which will provide new opportunities in diagnosis and treatment protocol.

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