

Effect of Scaling and Root Planing on Salivary Alkaline Phosphatase and Acid Phosphatase in Patients with Chronic Periodontitis

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Abstract

Background: Chronic periodontitis affects the supporting structures of the dentition, namely, the gingiva, periodontal ligament, cementum, and alveolar bone; it arises from interactions between the host and around 700 bacterial taxa in the subgingival microbiota, and the host responses include production of different enzymes that released by stromal, epithelial, inflammatory, or bacterial cells. **Objective:** This study aimed to evaluate the effect of scaling and root planing on the activity level of salivary alkaline and acid phosphatase (ACP) in patients with chronic periodontitis. **Materials and Methods:** The study was conducted on 60 patients, 40 were with chronic periodontitis and 20 were controlled with healthy periodontium. Scaling and root planing were conducted for periodontitis patients, and saliva samples were collected at baseline before treatment and after 2, 4, and 6 weeks of periodontal therapy and from control patients to estimate the activity levels of alkaline and ACP. **Results:** Scaling and root planing resulted in significant reduction of plaque index, gingival index, bleeding on probing, pocket depth, and clinical attachment level after 4 weeks of treatment ($P < 0.01$) and also reduction of activity levels of alkaline and ACP after 2 weeks of treatment and continued into 4 weeks as compared to baseline before treatment ($P < 0.01$, $P < 0.05$). **Conclusion:** The activity levels of both enzymes were reduced gradually after scaling and root planing due to the reduction of inflammatory reaction and healing of periodontal tissue, so both enzymes can be used as a biomarker for periodontitis diagnosis, treatment responses, and follow-up.

Keywords: Acid phosphatase, alkaline phosphatase, chronic periodontitis, enzyme

INTRODUCTION

Chronic periodontitis is an infectious disease that results in inflammation of the protective tissues of the teeth and the progressive loss of connective tissue and alveolar bone. It is the most frequently found periodontal pathology, and although its occurrence normally involves adult individuals, periodontitis can appear at any age.^[1] The diagnosis of periodontal disease is accomplished through clinical periodontal parameters including plaque index (PI), gingival index (GI), probing pocket depth (PPD), and Clinical attachment loss (CAL); however, the analysis of saliva can contribute to the diagnosis and prognosis of the disease.^[2] Numerous markers in saliva such as intracellular enzymes (creatine kinase, lactate dehydrogenase LDH, aspartate aminotransferases ASTs and alanine aminotransferases, gamma glutamyltransferase, alkaline phosphatase ALP, and acid phosphatase ACP) have been proposed as a diagnostic test for periodontal disease and

appear to be useful to test the activity of periodontal disease or to measure the effectiveness of periodontal therapy. LDH and AST can help to monitor the progression of periodontal disease.^[2-4] These enzymes are indicators of high level of cellular damage of periodontal tissue and their increased activity in gingival crevicular fluid (GCF), and saliva occurred as a consequence of their increased release from the damaged cells of soft tissues of the periodontium and reflected metabolic changes in the inflamed gingiva.^[2-4]

The analysis of these enzymes in salivary secretion can contribute to clarification of the pathogenesis and to improvement of making a prompt diagnosis of the periodontal

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DOI:
10.4103/MJBL.MJBL_48_18

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How to cite this article: Mohammad CA, Aziz HW. Effect of scaling and root planing on salivary alkaline phosphatase and acid phosphatase in patients with chronic periodontitis. Med J Babylon 2018;15:186-90.

disease.^[5] ALP is a calcium- and phosphate-binding protein and a phosphor-hydrolytic enzyme and is considered to be an important indicator of bone formation and is a phenotypic marker for osteoblast cells, submandibular and minor salivary glands, as well as in desquamated epithelial cells, leukocytes, and bacteria from dental plaque.^[6]

The presence of ALP in the saliva and in GCF is usually indicative of inflammation and/or destruction of the periodontal tissues, and the level of ALP is positively correlated with the severity of the periodontal disease.^[7] ALP enzyme is found in many cells of the periodontium including neutrophils, osteoblasts, and fibroblasts and is released during inflammation from polymorphonuclear neutrophils, by osteoblasts during bone formation and by fibroblasts during periodontal regeneration, presenting dual involvement in the process of periodontal inflammation and healing/regeneration. The sample sources through which ALP can be identified and estimated potentially include GCF, saliva, and serum.^[8,9] ALP could also have a bacterial origin and play a role in the formation of a pathological pocket.^[10]

ACP is a lysosomal enzyme and has high activity in bone-resorbing cells such as osteoclast and macrophages,^[11] it catalyzes a variety of challenging hydrolytic enzymes that occur in multiple molecular forms with lysosomes of cells from a variety of tissues,^[8,11] and it could also have a bacterial origin and play a role in the formation of a pathological pocket.^[10] A study on the structural and immunological work of ACP in the periodontal tissue revealed that ACP associated with bone resorption and high activity was observed in the osteoclasts of bones.^[5]

This study aimed to evaluate the effect of scaling and root planing clinically; throughout measurement, the mean value of PI, GI, bleeding on probing (BOP), PPD, and CAL after 4 weeks of periodontal therapy and compare them with baseline before treatment, and chemically throughout measurement, the activity level of salivary alkaline and ACP enzymes after 2, 4, and 6 weeks of periodontal therapy and compare them with baseline before treatment.

MATERIALS AND METHODS

Setting and time

The study was carried out in periodontic and biochemical laboratories of Basic Science Departments/College of Dentistry/Hawler Medical University/Erbil city, Iraq. The study period was started from April 2017 to December 2017.

Subjects

A clinical comparative study was conducted on 40 patients with chronic periodontitis and 20 controlled adult volunteers of both sexes with an age of more than 30–50 years. All the patients met the following inclusion criteria: had more than 20 teeth, were systemically healthy, no history of clinical periodontal treatment or drug intake for the last 6 months. Patients who were smokers, alcoholic, pregnant, lactating, or postmenopausal females, or others on estrogen therapy

were excluded. The study protocol was reviewed and approved by the Institutional Ethical Committee of College of Dentistry/Hawler Medical University, and informed consent was signed by all participants. The volunteers in the controlled participants showed healthy periodontium with no clinical evidence of gingivitis or chronic periodontitis, without gingival inflammation (GI = 0 and BOP = 0) or periodontitis (pocket depth [PD] ≥ 4 = 0 and clinical attachment loss [CAL] = 0), and saliva was collected from them only to investigate the normal mean value of ALP and ACP, while the volunteers in chronic periodontitis patients had two periodontal pockets depth of ≥ 4 mm in each quadrant and clinical attachment loss of ≥ 3 mm in one or more sites in two or more teeth.

Intraoral clinical examination

The clinical periodontal examination was carried out for all participants at the base line and after 4 weeks of periodontal therapy by double blinded technique after salivary sample collection. The clinical periodontal parameters included; probing pocket depth (PPD) which was assessed by insert gently calibrated William's periodontal probe from gingival margin to the base of sulcus or pocket at four surfaces of each tooth^[12], clinical attachment level (CAL) was assessed by measure the distance from cemento-enamel junction CEJ to the base of pocket by a calibrated William's periodontal probe^[12], plaque index (PI) was assessed by measuring the thickness of plaque for four surfaces of the examined tooth and given a score from 0–3^[13], gingival index (GI) was identified by measuring the extent and severity of gingival inflammation throughout inspection by naked eyes and by gentle probing using William's periodontal probe for four gingival surfaces of the examined tooth and given a score from 0–3^[14,15], bleeding on probing (BOP) was performed by running periodontal probe (William) gently along the inner surface wall of gingival sulcus and bleeding was noted after 30 seconds as absent (-) or present (+).^[16] Patients with periodontal disease were under conventional periodontal treatment consisting of motivation, oral hygiene instructions, scaling (supra and subgingival), and root planing only.

Saliva sample collection

Five milliliters of unstimulated saliva samples was collected from all 60 patients at 9–10 a.m. The patients were in a seated position with the head inclined forward, and spitting method was used for collection of unstimulated saliva from patients with chronic periodontitis at baseline before treatment and after 2, 4, and 6 weeks of periodontal treatment and also from controlled healthy patients. The saliva was collected in sterilized plastic test tube during 5 min period, immediately centrifuged at 3000 rpm for 20 min to obtain clear supernatant, and finally, stored at -20°C for later estimation of ALP and ACP.

Alkaline and acid phosphatase enzymes

Both ALP and ACP were estimated using BIOLABO kits, ALPBIOLABO SAS, France, and ACPBIOLABO SAS, France, respectively, which used for estimation of body fluid, and the procedure was performed according to the manufacturer's instructions.

Statistical analysis

Data were presented as mean and standard deviation. The data were statistically analyzed using Statistical Package for Science Services SPSS (version 22) by means of the student *T*-test. $P < 0.05$ was accepted as statistically significant.

RESULTS

A total of 60 participants comprising 22 females and 38 males with an age of 30–50 years were enrolled in this study, and the mean age for female and male was (34.27 ± 4.00).

Regarding clinical results, Table 1 reveals a significant reduction in the mean value of plaque score in patients with chronic periodontitis after 4 weeks of treatment as compared to its mean value at baseline before treatment ($P \leq 0.01$). The mean decrease in the mean value of PLI score was from 1.60 ± 0.37 at baseline to 0.9 ± 0.38 after 4 weeks of treatment.

For GI, there was also a significant reduction in the mean value of GI score after 4 weeks of treatment (1.11 ± 0.46) as compared to its mean value at baseline before treatment (1.74 ± 0.42) ($P \leq 0.01$).

For PD, there was also a significant reduction in the mean value of PD from 4.48 ± 0.32 mm at baseline to 3.52 ± 0.52 mm after 4 weeks of treatment ($P \leq 0.01$). For CAL, the result showed a significant reduction in the mean value of CAL from 3.47 ± 0.43 mm at baseline to 1.93 ± 0.61 mm ($P \leq 0.01$). Finally, for BOP, the result showed a significant reduction in the mean values of BOP after 4 weeks of treatment ($19.10 \pm 13.42\%$) as compared to its mean value at baseline before treatment ($73.15 \pm 19.02\%$).

Regarding biochemical results, Table 2 shows the mean activity levels of salivary ALP and ACP in patients with chronic periodontitis and the comparison between the mean activity levels of both enzymes at baseline before treatment and after different time intervals of 2, 4, and 6 weeks of periodontal treatment using paired sample *t*-test and showed that the mean activity level of salivary ALP was significantly reduce after 2 weeks of treatment (9.92 ± 0.77 IU/L) as compared to its mean value at baseline before treatment (19.42 ± 5.24 IU/L) ($P < 0.01$).

This decrease was continued into 4 weeks after treatment (9.50 ± 3.54 IU/L) with significant differences with its mean value at baseline before treatment (19.42 ± 5.24 IU/L) ($P < 0.01$), and then, the mean value of ALP increases after 6 weeks of treatment (27.72 ± 4.75 IU/L) with significant differences with its mean value at baseline before treatment (19.42 ± 5.24 IU/L) ($P < 0.01$).

Regarding ACP, the result showed that the mean activity level of ACP was significantly highly reduced after 2 weeks of treatment (28.57 ± 2.76 IU/L) as compared to its mean value at baseline before treatment (80.16 ± 5.42 IU/L) ($P < 0.01$) and slightly increased after 4 weeks of treatment (42.06 ± 2.42 IU/L) with significant differences with its mean value at baseline before treatment (80.16 ± 5.42 IU/L) ($P < 0.05$), and then,

Table 1: Comparison between the mean values of plaque index, gingival index, probing pocket depth, clinical attachment loss, and bleeding on probing in patients with chronic periodontitis before and after 4 weeks of treatment

Index	Time	Mean $\pm$ SD	n	P	Significance
PI	Before	1.60 $\pm$ 0.37	40	<0.01	Significant
	4 weeks	0.9 $\pm$ 0.38	40		
GI	Before	1.74 $\pm$ 0.42	40	<0.01	Significant
	4 weeks	1.11 $\pm$ 0.46	40		
PPD (mm)	Before	4.48 $\pm$ 0.32	40	<0.01	Significant
	4 weeks	3.52 $\pm$ 0.52	40		
CAL (mm)	Before	3.47 $\pm$ 0.43	40	<0.01	Significant
	4 weeks	1.93 $\pm$ 0.61	40		
BOP (%)	Before	73.15 $\pm$ 19.02	40	<0.01	Significant
	4 weeks	19.10 $\pm$ 13.42	40		

PI: Plaque index, GI: Gingival index, PPD: Probing pocket depth, CAL: Clinical attachment loss, BOP: Bleeding on probing, SD: Standard deviation

Table 2: Mean activity levels of salivary alkaline and acid phosphatase enzymes (alkaline phosphatase and acid phosphatase) in patients with chronic periodontitis at baseline before and after 2, 4, and 6 weeks of treatment

Enzymes	Time	n	Mean $\pm$ SD (IU/L)	P	Significance
ALP	Baseline	40	19.42 $\pm$ 5.24	<0.01	Significant
	2 weeks	40	9.92 $\pm$ 0.77		
	4 weeks	40	9.50 $\pm$ 3.54		
	6 weeks	40	27.72 $\pm$ 4.75		
ACP	Baseline	40	80.16 $\pm$ 5.42	<0.01	Significant
	2 weeks	40	28.57 $\pm$ 2.76		
	4 weeks	40	42.06 $\pm$ 2.42		
	6 weeks	40	84.72 $\pm$ 3.96		

ALP: Alkaline phosphatase, ACP: Acid phosphatase, SD: Standard deviation

the increase in the mean activity level of ACP continued into 6 weeks after treatment (84.72 ± 3.96 IU/L) with significant differences with its mean value at baseline before treatment (80.16 ± 5.42 IU/L) ($P < 0.01$).

Table 3, shows high significant differences in the mean activity levels of salivary ALP and ACP in chronic periodontitis patients (19.42 ± 5.24) (80.16 ± 5.42) IU/L at base line before treatment as compared to the their mean activity levels in normal healthy patients (13.32 ± 1.41) (44.02 ± 7.93) IU/L respectively $P < 0.001$, also significant differences were seen in the mean activity levels of both ALP and ACP in chronic periodontist patients after 2 weeks of treatment (9.92 ± 0.77) (28.57 ± 2.76) IU/L as compared to their mean activity values in normal healthy patients (13.32 ± 1.41) (44.02 ± 7.93) IU/L respectively $P < 0.001$, finally significant differences in the mean activity levels of ALP in saliva of chronic periodontitis patients after 4 and 6 weeks of treatment (9.50 ± 3.54) (27.72 ± 4.75) IU/L as compared to its mean activity values in normal healthy patients (13.32 ± 1.41 IU/L) $P < 0.001$, while for ACP, non-significant differences in the mean activity values of ACP in saliva of patients with chronic periodontitis after 4 weeks of treatment

Table 3: Comparison between normal healthy and chronic periodontitis patients at different time intervals in relation to alkaline and acid phosphatase enzymes (alkaline phosphatase and acid phosphatase)

Enzymes	Groups	Mean±SD (IU/L)	n	t-test	P
ALP	Normal	13.32±1.41	20	<0.001	Significant
	Baseline	19.42±5.24	40		
	Normal	13.32±1.41	20	<0.001	Significant
	2 weeks	9.92±0.77	40		
	Normal	13.32±1.41	20	<0.001	Significant
	4 weeks	9.50±3.54	40		
	Normal	13.32±1.41	20	<0.001	Significant
ACP	6 weeks	27.72±4.75	40		
	Normal	44.02±7.93	20	<0.001	Significant
	Baseline	80.16±5.42	40		
	Normal	44.02±7.93	20	<0.001	Significant
	2 weeks	28.57±2.76	40		
	Normal	44.02±7.93	20	0.33	Nonsignificant
	4 weeks	42.06±2.42	40		
	Normal	44.02±7.93	20	<0.001	Significant
	6 weeks	84.72±3.96	40		

ALP: Alkaline phosphatase, ACP: Acid phosphatase, SD: Standard deviation

(42.06±2.42 IU/L) were seen as compared to its mean values in normal healthy patients (13.32 ± 1.41 IU/L) $P < 0.001$, while for ACP, nonsignificant differences in the mean value of ACP in saliva of patients with chronic periodontitis after 4 weeks of treatment (42.06 ± 2.42 IU/L) were seen as compared to its mean activity values in normal healthy patients (44.02±7.93 IU/L) (P 0.33), and finally after 6 weeks of treatment the mean activity levels of ACP increased significantly into (84.72±3.96IU/L) with significant differences with its mean values in saliva of normal healthy patients (44.02±7.93 IU/L) ($P < 0.001$).

DISCUSSION

Clinically, the results showed that scaling and root planing had a significant positive effect in reduction of gingival inflammation and healing of periodontal tissue throughout reduction of bacterial plaque accumulation, PI, gingival inflammation (GI and BOP), PD, and clinical attachment loss (CAL) after 4 weeks of treatment; this may be due to that scaling and root planing disrupt the subgingival plaque biofilm, allowing shift in the microbial population to those more commonly associated with health.^[17,18] These results are in agreement with other studies which concluded that scaling and root planing by using manual or sonic driven scalers in subgingival pockets resulted in a profound shift in the composition of subgingival microbial flora and by removing the endotoxins in the subgingival areas, healing of the tissues can occur, and a reduction in BOP, probing depth, and CALs can be attained after 4 weeks of periodontal therapy.^[17,18]

Another study concluded that nonsurgical periodontal therapy resulted in elimination of pathogenic microbial plaque and contributing factors for gingival and periodontal diseases which

result in halting the progression of the periodontal disease and it returns to a state of health;^[19,20] also, the re-evaluation should occur after 4 weeks of scaling and root planing, because during this time, both epithelial and connective tissue healing occurred, which consists of formation of long junctional epithelia attached to the root surface rather than new connective tissue attachment;^[21] the gradual reductions in inflammatory cell infiltration, crevicular fluid flow, and repair of connective tissue resulted in decreased clinical signs of inflammation with less redness and swelling, so the effect of scaling and root planing is effective and reliable;^[3,19] in the same line with our study, studies ranging from 1 month to 2 years in length demonstrate up to 80% reduction in BOP and mean of probing depth reduction of 2–3 mm, and other study demonstrated that the number of pockets 4 mm in depth or greater was reduced by 52%–80%.^[19,21]

Biochemically, the results showed that the activity of the examined salivary ALP and ACP enzymes was high in patients with chronic periodontitis as compared with normal healthy patients, and their activity started to decrease significantly after 2 and 4 weeks of treatment as compared to patients with periodontitis before treatment. This increase in the activity of salivary ALP and ACP in periodontitis patients than normal healthy patients, is an indicator of high levels of cellular damage of periodontal tissue and increased release of both enzymes from damaged cells of the soft tissue of periodontium into GCF and consequently into saliva.

Results of this study were in agreement with those obtained by several authors^[2-4,7,22,23] who reported that salivary ALP and ACP associated with cell death and injury and the changes in enzymatic activity of salivary ALP and ACP reflect metabolic changes in the gingiva and periodontium during inflammation;^[3,4] other studies identified that the increased activity of ACP and ALP during periodontitis might be a consequence of destructive processes in the alveolar bone and is associated with the advanced stages of development of periodontal disease and determined a positive correlation between the activity of ALP and the percentage of the alveolar bone loss;^[4,23] a study reported that the increased activity of ACP, especially ALP in periodontitis patients than healthy, indicated that the pathological destructive process affected the alveolar bone. This also indicated that the periodontal disease has significantly advanced; thus, the prognosis is much worse;^[24] another studies concluded that the activity of these enzymes in saliva could be useful for the assessment of efficiency of changing the therapy in curing periodontal disease.^[3,4,23]

In the same line with our results, other studies reported that both enzymes reduced significantly into after 4 weeks of treatment due to tissue repair,^[25] and other study showed that scaling was effective in decreasing the activities of both enzymes, which may be due to the role of scaling in removal of plaque which consists mainly of bacteria, which may be a source for ALP and ACP in GCF and consequently into saliva; therefore, their release was decreased after treatment.^[5,26]

Other studies reported that the activity of ALP^[5,7,24] and ACP were increased in periodontitis patients as compared to

healthy^[22] non periodontitis and after therapy their activities restored to that found in healthy persons, and concluded that the increased mean value of ACP in periodontitis patients due to that chronic periodontitis is associated with elevated systemic inflammatory markers and production of several lysosomal enzymes, and since ACP is an intracellular enzyme present in most of the tissues and is an indicator of increased cellular damage in the soft tissues of the periodontium and inflamed gingival tissues,^[22] other study reported that the increased levels of ACP in periodontitis were more likely to be associated with the metabolic disorder.^[27]

Another study showed that the level of ACP enzyme in the gingival tissue correlates with the severity of periodontitis.^[11,28] In contrary, another study found nonsignificant difference between periodontitis and nonperiodontitis patients in relation to ACP.^[22] Furthermore, our study showed that both enzymes increased after 6 weeks of treatment which may be due to return habit of neglect teeth brushing and recurrence accumulation of bacterial plaque (important source of both enzymes) and its associated gingival inflammation and then periodontal tissue destruction.

CONCLUSION

The present study concluded that chronic periodontitis changes the activities of salivary ALP and ACP enzymes, which was represented by increased the activity levels of both enzymes, and after treatment, the activity of both enzymes was significantly reduced into even after 4 weeks of treatment which is probably as a result of reduction of bacterial plaque accumulation, periodontal inflammation, and periodontal tissues repair. Based on these results, it can be assumed that the activity of these enzymes in saliva, as biochemical markers for periodontal tissue damage, may be useful in diagnosis, prognosis, and evaluation the effects of periodontal therapy on periodontal disease so can be used as biomarkers for periodontitis treatment responses and follow-up.

Acknowledgment

The authors would like to thank Dr. Bakhtiar M. Ahmed, the head of Basic Science Department/College of Dentistry, Hawler Medical University, for giving us the chance to perform the biochemical study under his supervision.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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