

Estimation of GCF Alkaline Phosphatase Levels in Patients with Aggressive Periodontitis and Chronic Periodontitis Before and after Periodontal Therapy - A Clinico-Biochemical Study

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A B S T R A C T

Introduction: Periodontal diseases are a very common cause for damage to periodontium and loss of teeth. Tooth mobility, pathological migration and alveolar bone loss are common sequelae. Slowly progressing marginal periodontitis also called as chronic periodontitis and aggressive periodontitis are the two well-known types. Alkaline phosphatase activity in gingival crevicular fluid (GCF) has been suggested as a useful marker for early diagnosis of periodontal disease as well as for its follow up of its progress upon treatment. Based on this observation, a study has been conducted in patients with chronic and aggressive periodontitis.

Material and Methods: Gingival index, probing pocket depth and alkaline phosphatase activity in GCF were observed in patients with aggressive and chronic periodontitis before and after periodontal therapy.

Results: On Comparison between pre and post treatment values of GCF alkaline phosphatase between both aggressive and chronic periodontitis, the mean difference was statistically highly significant ($P < 0.001$) using paired t test.

Conclusion: It was concluded that the mean alkaline phosphatase levels in the patients with aggressive and chronic periodontitis were significantly decreased after periodontal therapy.

Keywords: Periodontitis, Alkaline Phosphatase, Gingival Crevicular Fluid, Aggressive Periodontitis, Chronic Periodontitis

INTRODUCTION

Inflammatory, degenerative and proliferative diseases of the periodontium lead to changes in the quality and quantity of its connective tissue constituents.¹ The destruction of the dental apparatus in periodontal disease generally results from an inflammatory process and occurs as early and continuing events and in cycles of exacerbation and quiescence.² Inflammation is characterised by the migration of phagocytic cells from peripheral circulation to the site of infection. These cells especially granulocytes, contain numerous proteolytic enzymes which degrade plasma proteins and components of the extracellular matrix and thereby damage host tissues.³ Accurate prediction and diagnosis of periodontal disease activity would aid in prevention and treatment of periodontal attachment loss.⁴ The biochemical changes associated with inflammatory process precede the clinical signs of disease. Therefore analysis of GCF alkaline phosphatase activity has received considerable interest.⁵

Alkaline phosphatase is a membrane bound glycoprotein produced by many cells within the area of periodontium and gingival crevice. It is a lysosomal enzyme that is released into crevicular fluid during inflammatory process by degradation of polymorphonuclear leucocytes, host tissue injury and bacterial degradation. It is associated with inflammation and hence alteration in its levels in GCF indicates initiation and progression of periodontitis.^{6,7} Based on this observation, a study has been conducted in patients with chronic and aggressive periodontitis.

MATERIAL AND METHODS

The study was conducted in the Department of Periodontia, Government Dental College and Hospital, Afzalgunj, Hyderabad after ethical clearance. The sample consisted of 20 patients belonging to both sexes and with age ranging from 18- 55 years. The patients were divided into two groups of 10 each as aggressive periodontitis group (Group A) and chronic periodontitis group (Group B).

B). patients below 18 years and greater than 55 years were not taken.

Inclusion criteria: Group A consisted of 10 patients with ages ranging from 17 - 22 years. Group B consisted of 10 patients with ages ranging from 35-55 years. Group assignment was based on mean gingival index (GI), Probing Depth (PD), radiographic evidence of bone loss of the sampling sites and the number of sites affected. The patients had to have gingival index greater than or equal to 1.0, probing depth greater than or equal to 5.0 mm, radiographic evidence of 30-60% bone loss and 2-3 affected teeth per quadrant.

Exclusion criteria: Patients with a history of systemic condition which might influence the course of periodontal disease or future periodontal therapy, patients on anti-inflammatory or antibiotic drug therapy during the previous 3 months, patients with a history of past periodontal therapy, orthodontic therapy or prosthetic appliance during previous 6 months and patients with teeth fewer than 20 were excluded.

Study design: At the base line examination case history and clinical parameters were recorded. At the second appointment, i.e. 2 weeks after the baseline examination GCF was collected and clinical parameters were recorded. Periodontal therapy was started in the patients. At the third appointment, i.e. 2 weeks after the completion of periodontal therapy GCF fluid collection was done and the clinical parameters were recorded (figure 1-5).

Armamentarium used were mouth mirror, William's periodontal probe, tweezers, dry cotton for isolation, air syringe, Whatman 3 mm chromatography paper, graduated metallic scale, blade, test tubes, aluminium foil, incubator, volumetric pipettes, spectrophotometer with cures, reagents i.e. disodium phenyl phosphate (analarbiochemical, BDH chemicals limited. Poole England), alkaline buffer- carbonate bicarbonate buffer, 0.5 N sodium hydroxide, 4.2% sodium bicarbonate, 0.6% 4 amino antipyrine, 2.4% potassium ferricyanide, stock standard phenyl solution, working standard phenol solution, distilled water (figure-6).

Methodology: The intracrevicular method of Brill and Krasse, 1958 was used for gingival crevicular fluid collection.⁸ The area of the mouth to be sampled was isolated with cotton rolls, the supragingival plaque was carefully removed with a curette type scaler and dried with a gentle stream of air with an air syringe. A pre-cut whatman 3 mm chromatography paper strip was gently inserted into the crevice until minimum resistance was felt and left in place till visibly wet. An interval of 10 minutes was allowed between collections of each set to permit normalization of GCF yield. The strips were then stored at -20 degree Celsius till biochemical analysis but not more than 24 hours after collection of the samples.

Alkaline phosphatase was estimated by colorimetric method of Kind and King, 1954 using 4 amino phenazone. The principle was the enzyme present in the sample reacts with the substrate disodium phenyl phosphate and hydrolysed with the liberation of phenol and sodium phosphate. The hydrolysis product, phenol is condensed with 4- amino antipyrine and then oxidised with alkaline ferricyanide to give red complex, which is measured photometrically at 540 nm. The estimation of phenol is equivalent to the amount of alkaline phosphatase present in the sample spectrophotometer capable of providing an accurate absorbance measurement at 540 nm is suitable. Water bath- 37 degree Celsius. The entire procedure from the intracrevicular placement of 3mm chromatography paper strip till the determination of enzyme activity took about 2 hours. The various findings were recorded using a designed proforma.

STATISTICAL ANALYSIS

The results of the study were subjected to statistical analysis by applying analysis of variance (ANOVA), multiple range test and paired t test.

RESULTS

The mean GCF alkaline phosphatase levels of Group A when collected initially was 191.90 K.A. units with a standard deviation of 22.63 and that of group B was 296.65 K.A. Units with a standard deviation of 83.11. When these initial GCF Alkaline phosphatase levels of Group A and Group B were compared, group B that is patients with chronic periodontitis showed increase in the mean alkaline phosphatase levels which is significant statistically at $P < 0.001$. The mean GCF alkaline phosphatase values post treatment in group A was 147.98 with a standard deviation of 56.58 and group B was 178.14 with standard deviation of 51.47. On comparison between pre and post treatment values of GCF alkaline phosphatase in group A the mean difference was statistically highly significant

Treatment	No. of Subjects	Mean	Standard Deviation
Pre - treatment	10	191.90	22.63
Post - treatment	10	147.98	56.58
P < 0.01			
Table-1: the mean \pm standard deviation of GCF alkaline phosphatase levels - pre and post treatment in patients with aggressive periodontitis i.e, group A			

Treatment	No. of Subjects	Mean	Standard Deviation
Pre - treatment	10	296.65	83.11
Post - treatment	10	178.14	51.47
P < 0.001			
Table-2: The mean \pm standard deviation of GCF alkaline phosphatase levels - pre and post treatment in patients with chronic periodontitis i.e, group B			



Figure-1: Aggressive periodontitis Pre - treatment, **Figure-2:** Chronic periodontitis Pre – treatment



Figure-3: Aggressive periodontitis Post - treatment; **Figure-4:** Chronic periodontitis Post – treatment

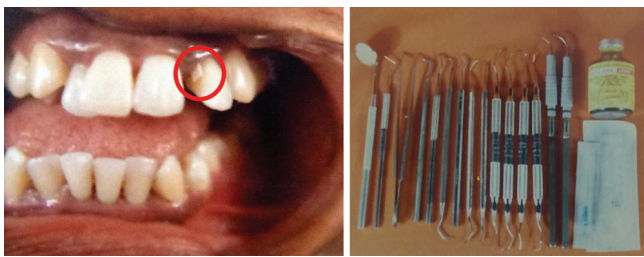


Figure-5: GCF collection by 3 mm chromatography paper strip; **Figure-6:** Armamentarium

($P < 0.01$) using paired t test (Table 3). On Comparison between pre and post treatment values of GCF alkaline phosphatase in group B the mean difference was statistically highly significant ($P < 0.001$) using paired t test (Table 4).

DISCUSSION

Periodontitis in humans is an inflammatory process and the microorganisms or their products and components are the driving force behind the observed tissue destruction.⁹ The first description of alkaline phosphatase was given in the literature by Suzuki U Yoshimura and Takaisi K in 1907. The development of the colorimetric method in 1924 helped martland and his associates to demonstrate the extent of alkaline phosphatase in human blood. Alkaline Phosphatase is the orthophosphoric monoester polyhydrolase and it belongs to the class hydrolases and the sub class esterases. It is a nonspecific phosphate that effects the hydrolysis of monophosphoric acid esters at an alkaline pH.¹⁰

Alkaline phosphatase is demonstrable at sites of high metabolic activity both in normal and diseased tissues and is not seen in sites of low metabolic activity such as adipose tissue.¹¹ The physiological significance of alkaline phosphatase is in the process of calcification and ossification in certain sites of inflammatory reaction,

glandular secretion, absorption and resorption by epithelium, histodifferentiation and transmembrane solute passage. Increased alkaline phosphatase activity has been noticed in diseases manifesting active proliferation as seen in granulation tissue, inflammatory infiltrates and moderate to intense activity has been noticed in early stages of connective tissue proliferation such as scar tissue.¹¹

The normal value of serum alkaline phosphatase in an adult is 3-13 K-A units%. Alkaline phosphatase activity is reported to be involved in calcification, in differentiation of both osteoblasts and chondroblasts from their mesenchymal origin¹², and in the formation of mucopolysaccharides of the ground substance.¹³ Alkaline phosphatase is a membrane bound glycoprotein produced by many cells in the area of periodontium and gingival sulcus. The distribution of alkaline phosphatase in gingival tissues was studied and it was reported that alkaline phosphatase is present in the endothelial cells, in the capillary walls and possibly in the fibers of the connective tissue.¹⁴ Alkaline phosphatase appears to related to the formation of connective tissue since the great enzymic response was observed when fibroblastic proliferation and fibrogenesis reached their greatest activity. The increase in alkaline phosphatase is due to collagen synthesis in reparative process.¹⁵ It has also been reported that the enzyme is present in keratinized and parakeratinized surface layers of gingiva. The enzyme activity especially in the gingival connective tissue adjacent to the epithelial tissue plays a role in keratinization.¹⁶ It was reported in a histographic study of alkaline phosphatase in periodontal tissue that alkaline phosphatase positive cells are present in the apical portion of epithelial cuff and the association of alkaline phosphatase enzyme with Vonkorff fibres. In the histographic study of alkaline phosphatase in periodontal tissue, it is noticed that this enzyme appears in gingival blood vessels, periosteum and periodontal fibres but not in epithelium.¹⁷

Carranza and cabrini studied the distribution of alkaline phosphatase in periodontal tissues in case of gingivitis and periodontitis. In the periodontal pocket, large amount of alkaline phosphatase was found in the granulation tissue of the lateral wall and of the bottom of the pocket, and gingival fibers at the point of their insertion. The sources of alkaline phosphatase are polymorphonuclear leucocytes, bacteria within the dental plaque and osteoblasts and fibroblasts.¹⁸ Ishiwaka and Cimasoni in 1970 first recognised the potential of alkaline phosphatase as an important biochemical component of gingival crevicular fluid. They carried out quantitative analysis of alkaline phosphatase from crevicular exudate in patients with periodontal diseases. The concentration of alkaline phosphatase was found to be significantly correlated with pocket depth and mean percentage of bone loss.¹⁹ Curtis

et al suggested that the fluid accumulates at the gingival margin, it will contain potential markers derived not only from the host tissue and serum but also from subgingival microbial plaque and thus an extremely broad range of candidate molecules may be investigated.⁷

Chapple et al have investigated GCF alkaline phosphatase levels in health and in the presence of gingivitis. In gingival health, there was a site specific pattern of alkaline phosphatase concentration with higher enzyme concentrations around the upper and lower anterior teeth. Furthermore, clinically normal sites that had been subjected to different levels of plaque control produced significantly different alkaline phosphatase levels. This indicated that the biochemical components of GCF may be used to measure sub clinical inflammatory status. The ratio of GCF to serum alkaline phosphatase varied from 6:1 to 11:1 suggesting that the major source of the enzyme is from local production. There was no significant relationship between total GCF alkaline phosphatase and plaque levels of the enzyme and analysis of the plaque within the study group demonstrated very low levels of alkaline phosphatase indicating that the enzyme is likely to be largely derived from the periodontal tissues.²⁰

The increase in alkaline phosphatase in inflamed periodontal tissues indicate a metabolic reaction of the enzyme against the tissue changes caused by inflammation. Alkaline phosphatase concentration was shown to be positively associated with periodontal disease activity²¹ and its activity is measured as possible indicators of gingival inflammation and bone metabolism. In a study of the distribution of alkaline phosphatase in periodontal tissues in case of gingivitis and periodontitis, the amount of enzyme is greatly increased when inflammation occurs.¹⁸ In a study it is observed that the activity of alkaline phosphatase in elderly is lower than in young because the slow metabolic activity of gingival cells with advancing age results in decreased enzyme activity.²² Nakashima et al, reported from their study that total amounts of alkaline phosphatase were higher in periodontitis as compared to healthy and gingivitis sites.²³

There are abundant Polymorphonuclear leucocytes in the site of periodontal inflammation and they are the prime source of GCF alkaline phosphatase.^{24,25} Kunjappu et al, reported from their study that the decrease in inflammation as a result of mechanical plaque control methods such as scaling and root planning results in significant decrease if GCF alkaline phosphatase values.²⁶

In the present study, on comparison of pre and post treatment values in group A i.e., aggressive periodontitis cases the mean difference of GCF alkaline phosphatase was statistically significant and comparison of pre and post treatment values of group B, i.e., chronic periodontitis cases the mean difference of GCF alkaline phosphatase was highly significant statistically. This indicates a reduction

in alkaline phosphatase levels after periodontal therapy in both the groups. This may be because of the removal of local irritational factors which results in reduction in inflammation upon treatment.

CONCLUSION

The alkaline phosphatase levels in GCF can be used as a marker for diagnosis and follow up to assess the health and pathology and changes on treatment of the periodontium. It can also be used in early detection of periodontal changes and can assess the efficacy and prognosis of the periodontal therapy.

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