

Estimation of GCF Alkaline Phosphatase Levels in Health and Periodontal Disease – A Clinico Biochemical Study

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ABSTRACT

Introduction: Periodontitis is an inflammatory disease that is a common cause for damage to gingiva and periodontal tissue and for the loss of teeth. Tooth mobility, pathological migration and alveolar bone loss are common sequelae of periodontitis. Two types of periodontitis known are Chronic and Aggressive type. In gingival crevicular fluid (GCF), the activity of Alkaline phosphatase has been considered as a useful diagnostic and prognostic marker of periodontal disease. Based on this observation, a study has been conducted in patients with chronic and aggressive periodontitis and their age and gender matched healthy controls to assess the ALP levels in GCF.

Material and methods: GCF alkaline phosphatase activity was observed in patients with aggressive and chronic periodontitis and their age and gender matched healthy controls.

Results: On Comparison between GCF alkaline phosphatase levels between both aggressive and chronic periodontitis and their age and gender matched controls, the mean difference was statistically significant ($P < 0.01$) when paired t test was used.

Conclusion: In patients with aggressive and chronic periodontitis, the mean alkaline phosphatase levels were increased significantly in comparison to healthy controls.

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

INTRODUCTION

Periodontal diseases which consist of a group of inflammatory diseases result from an interaction of periodontal microflora that colonizes the teeth and the multifaceted host response. The neutrophils present within the gingival sulcus which play a role in the innate cellular response, ingest the bacteria and secrete proteolytic enzymes and immunomodulatory compounds. The end result of this is the clinical manifestation of the disease which comprises of several signs and symptoms such as increasing probing pocket depth and radiographic evidence of bone loss. These effectively describe a periodontal history and have been used semi quantitatively to evaluate patients with periodontal disease but cannot indicate the present or future extent of periodontal destruction. Though the conventional methods to evaluate the periodontal tissues are usually sufficient to arrive at a diagnosis and enable effective clinical treatment, they also have limitations like detection of periodontal disease before it has caused destruction and difficulty in determination of activation of periodontal disease and its treatment.¹⁻⁶

Gingival Crevicular fluid (GCF) is a transudate that is formed when fluid excludes from the vessels of the microcirculation into the periodontal tissue and the gingival sulcus or pocket. It is derived from microbial dental plaque, host tissues, polymorphonuclear leukocytes, macrophages, plasma cells and serum, but it becomes an exudate in case of inflammation. A number of GCF constituents including host enzymes in particular have been shown to be diagnostic markers of periodontal health and disease. Alkaline phosphatase (ALP) is one of the first of the diagnostic markers identified in GCF.^{1,6,7}

ALP which is produced by various cells such as polymorphonuclear leucocytes, osteoblasts and fibroblasts present within the area of periodontium and gingival crevice is a membrane bound glycoprotein. It is not only a part of root cement formation and maintenance, bone homeostasis and normal periodontal ligament turnover but also is an important lysosomal enzyme released into GCF in inflammation by host tissue injury, degradation of polymorphonuclear leucocytes and bacterial degradation. Hence a variation in activity and levels of ALP in GCF indicates initiation and progression of periodontitis.^{1,8-12} Based on this observation a study was conducted to assess the levels of ALP in GCF in patients with periodontitis and in healthy controls.

MATERIAL AND METHODS

The study was conducted at the Department of Periodontics, Government Dental College and Hospital, Afzalgunj, Hyderabad after obtaining ethical clearance. The sample consisted of 40 patients belonging to both sexes and with age ranging from 18- 55 years, divided into four groups of 10 each as aggressive periodontitis group (Group A) and associated age and sex matched healthy controls (Group B) chronic periodontitis group (Group C) and associated age and sex matched healthy controls (Group D). Patients below 18 years and greater than 55 years were not included.

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Inclusion Criteria: Group A comprised of 10 aggressive periodontitis patients with ages ranging from 18 - 22 years. Group B consisted of 10 healthy controls with ages ranging from 18-22 years. Group C consisted of 10 chronic periodontitis patients with ages ranging from 35-55 years. Group D consisted of 10 healthy controls with ages ranging from 35-55 years. Group allocation 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 in group A and C had a gingival index ≥ 1.0 , probing depth ≥ 5.0 mm, radiographic evidence of 30-60% bone loss and 2-3 affected teeth per quadrant.

Exclusion Criteria: Patients who had a history of any systemic disease or condition which may have an influence on the course of periodontal disease, patients who have undergone periodontal therapy, patients on anti-inflammatory or antibiotic drug therapy in the past 3 months, patients with a history of orthodontic therapy or prosthetic appliance during previous 6 months and patients with teeth less than 20 were excluded.

Study design: Initial examination comprised of recording case history and clinical parameters. At the second appointment, i.e. 2 weeks after the baseline examination GCF was collected and clinical parameters were recorded. 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.

Methodology: For the collection of GCF, intracrevicular method of Brill and Krasse, 1958 was used.⁸ Cotton rolls were used to isolate the area of the mouth to be sampled, Curette and scaler was used to remove the supragingival plaque carefully and the area was dried with a gentle stream of air with an air syringe. A pre-cut Whatman 3 mm chromatography paper strip was placed gently into the crevice until minimum resistance was felt and left in place till visibly wet. To permit normalization of GCF yield, an interval of 10 minutes was allowed between collections of each set. The strips were then stored at -20°C until biochemical analysis but not for more than 24 hours after collection of the samples. Colorimetric method of Kind and King, 1954 which utilised 4 amino phenazone was used to estimate Alkaline phosphatase. The principle being the enzyme present in the sample reacts with the substrate disodium phenyl phosphate and hydrolysed with the release of phenol and sodium phosphate. The amount of phenol is equivalent to the amount of alkaline phosphatase present in the sample spectrophotometer providing an accurate absorbance measurement at 540 nm.

Group	No. of subjects	Mean	Standard Deviation	
A	10	191.90	22.63	
B	10	77.96	32.50	P < 0.01

Table-1:

Group	No. of subjects	Mean	Standard Deviation	
C	10	296.65	83.11	
D	10	54.28	6.02	P < 0.01

Table-2:

STATISTICAL ANALYSIS

The results of the study were statistically analysed using paired t test with the help of SPSS 2017.

RESULTS

In Group A, the mean GCF alkaline phosphatase level was 191.90 K.A. units with a standard deviation of 22.63, Group B was 77.96 K.A. units with a standard deviation of 32.50, Group C was 296.65 K.A. units with a standard deviation of 83.11 and that of Group D was 54.28 K.A. units with a standard deviation of 6.02. On comparison of the mean values of alkaline phosphatase between Group A and Group B, Group A showed a significant increase in the mean value which is statistically significant at $P < 0.01$. when Group C and Group D are compared, the Group C showed a significant increase in mean alkalie phosphatase levels which is statistically significant at $P < 0.01$. when Group A and Group C were compared, Group C showed an increase in mean alkaline phosphatase levels which was significant statistically at $P < 0.01$.

The table 1 represents the mean values of alkaline phosphatase of Group A and Group B of patients with standard deviation and p value.

The table 2 represents the mean values of alkaline phosphatase of Group C and Group D of patients with standard deviation and p value.

DISCUSSION

Periodontal disease is an inflammatory process initiated by the group of microorganisms and their products that colonise the teeth and the surrounding soft tissues and form the components of the tissue destruction. Periodontal disease manifests in the form of the clinical signs and symptoms like increased probing pocket depth, radiographically evident bone loss which are used to semiquantitatively evaluate the affected patients.^{6,13} The pupose of this study was to evaluate the correlation of ALP levels in GCF in periodontal disease and in healthy tissues and the result suggests that ALP is an important enzyme of GCF for evaluation of the status of periodontal tissues.

The ecology of the periodontal poket is determined by GCF which comprises of blood and gingival tissue derived molecules and products derived from the subgingival plaque. As it contains the products of inflammatory response it also determines the growth rate of subgingival microorganisms

and is a potential diagnostic or prognostic marker of periodontal disease. GCF collection is non-invasive, easy to perform and is site specific in the teeth. It can be collected by various methods depending on the nature of analysis. Due to the accessibility and ease of transport filter paper strips are most commonly preferred method of collection of GCF. Studies have previously reported that there is a significant increase in GCF production in the presence of periodontal disease or destruction and hence the volume can serve as an index for the extent of periodontal destruction.^{1,14-16}

Suzuki U Yoshimura and Takaisi K in 1907 first described Alkaline phosphatase in the literature. Martland and his associates demonstrated the extent of alkaline phosphatase in human blood after the development of the colorimetric method in 1924. Alkaline Phosphatase which belongs to the class hydrolases and the sub class esterases, is an orthophosphoric monoester polyhydrolase a nonspecific phosphate. At an alkaline pH, it affects the hydrolysis of monophosphoric acid esters.¹⁷ It is demonstrable at sites of high metabolic activity in normal and diseased tissues and not seen in sites of low metabolic activity like adipose tissue.¹⁸ In diseases involving active proliferation of granulation tissue and inflammatory infiltrates increased alkaline phosphatase activity has been noticed and in early stages of connective tissue proliferation such as scar tissue, moderate to intense activity of ALP has been observed.¹¹ In an adult, the normal value of serum ALP is 3-13 K-A units%. Alkaline phosphatase activity is observed in calcification, in differentiation of osteoblasts and chondroblasts from their mesenchymal origin, and in the formation of mucopolysaccharides of the ground substance.¹² ALP is a membrane bound glycoprotein produced by many cells in the area of periodontium and gingival sulcus. Alkaline phosphatase distribution in gingival tissues was reported to be present in the fibers of the connective tissue, endothelial cells and capillary walls. Since the great enzymic response was observed during fibroblastic proliferation and fibrogenesis, it has also been related to the formation of connective tissue. Its increase is due to collagen synthesis in reparative process.⁸ A histographic study of alkaline phosphatase in periodontal tissue was conducted which showed that the apical portion of the epithelial cuff comprised of alkaline phosphatase positive cells and also evaluated the association of alkaline phosphatase enzyme with Vonkorff fibres. It was also noticed that this enzyme appears in periodontal fibres, gingival blood vessels and periosteum but not in epithelium.^{8,19}

The distribution of ALP in periodontal tissues in gingivitis and periodontitis as studied by Carranza and Cabrini, is that ALP was found in large amounts in the granulation tissue of the lateral wall and of the bottom of the periodontal pocket and at the gingival fibers at the point of their insertion. It was also observed that polymorphonuclear leucocytes, bacteria within the dental plaque, osteoblasts and fibroblasts were the main sources of ALP.²⁰

GCF alkaline phosphatase level in health and in the presence of gingivitis was investigated by Chapple et al and it was concluded that alkaline phosphatase concentration

in gingival health was site specific with higher enzyme concentrations around the maxillary and mandibular anterior teeth. It was also observed that clinically normal sites that were subjected to different levels of plaque control produced significantly different alkaline phosphatase levels. The ratio of GCF to serum alkaline phosphatase ranged from 6:1 to 11:1 recommending that the major origin of the enzyme is from local production and there was no significant relationship between total GCF ALP and plaque levels of ALP and an analysis of the plaque revealed low levels of ALP recommending that the enzyme is derived from the periodontal tissues, hence the increase in alkaline phosphatase in periodontal disease indicate a metabolic reaction of the enzyme against the tissue changes caused by inflammation. The concentration of Alkaline phosphatase was shown to be positively associated with periodontal disease activity.^{21,22} Its activity is observed as possible indicator of gingival inflammation and bone metabolism and the quantity of enzyme is considerably elevated when inflammation occurs.²⁰ Similarly in the present study also it was observed that the alkaline phosphatase levels were increased in disease states than in healthy states.

CONCLUSION

The alkaline phosphatase levels in Gingival crevicular fluid can be used both as a diagnostic and prognostic marker along with the clinical signs and symptoms to assess periodontal disease and its progression. It can also be used in the early detection of gingival and periodontal disease states.

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