Research Paper

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Using an Enzyme As a Periodontal Disease Marker

Medical Science

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ABSTRACT

Background: The potential of alkaline phosphatase as an important diagnostic marker of gingival crevicular fluid (GCF) has been the subject to investigation since 1970s. Alkaline phosphatase is mainly released during migration of neutrophils to the site of infection. It is also present in bacteria within dental plaque, osteoblasts and fibroblasts. It has thus become important to elucidate whether GCF levels of alkaline phosphatase are potential measures of inflammatory activity going on in the adjacent periodontal tissues.

Objective: The aim of this study was to assess total activity of alkaline phosphatase in the GCF collected from healthy sites, sites with gingivitis and chronic adult periodontitis and to find correlation of ALP activity with plaque index, gingival index, bleeding index and probing pocket depth.

Material and methods: A total of 18 patients were equally divided into three groups viz. healthy sites (I), gingivitis (II), chronic periodontitis (III). Clinical parameters like plaque index, bleeding index, gingival index, probing pocket depth were recorded. The ALP level in GCF of all three groups was determined by spectrophotometric analysis.

Results: Total enzyme activity of ALP was significantly higher in periodontitis as compared to healthy and gingivitis sites, and was positively correlated with probing pocket depth.

Conclusion: Alkaline phosphatase can be considered as a periodontal disease marker as it can distinguish between healthy and inflamed sites.

Introduction

Periodontal diseases consist of a group of inflammatory diseases initiated by bacteria that colonize the teeth and infect their surrounding soft tissues. The end result of this infection is the clinical manifestation of disease which results in several distinct signs and symptoms. For the most part, these clinical signs and symptoms, including radiographic evidence of bone loss and increasing pocket depth, have been used in a semi-quantitative manner to evaluate patients afflicted with periodontal diseases [1]. Conventional methods of evaluating periodontal tissues are usually sufficient to arrive at a diagnosis and enable effective clinical treatment but they are associated with a few limitations.[2]

1. They do not detect presence of disease before it has caused destruction.
2. It is difficult to determine when the periodontal disease is active and calls for treatment
3. Inability to reflect even marked histological changes

An era of "enlightened diagnosis" has resulted that has spawned many new methodologies designed to improve patient care. These include Genetic susceptibility analysis, Microbiologic analysis, Biochemical analysis. Biochemical methods in periodontal diagnosis have centered primarily on gingival crevicular fluid (GCF) and its constituents.2

GCF is formed when fluid exudes from the vessels of the microcirculation into the inflamed periodontal tissue and into the sulcus or pocket. As the fluid traverses the inflamed tissue, it is thought to pick up enzymes and other molecules that participate in the destructive process, as well as products of cell and tissue degradation. A few of the choice contenders found in GCF are products of poly-morphonuclear leukocytes (PMNs), macrophages, and plasma cells. 1

The fluid, therefore, offers great potential as a source of inflammatory and immune markers, tissue breakdown products, enzymes of bacterial origin, host derived enzymes and their inhibitors. The biochemical markers in gingival crevicular fluid have been related to gingivitis, periodontitis and, most recently to periodontal disease activity. Analysis of gingival crevicular fluid therefore represents the most practical approach for the biochemical analysis of the host response in periodontal disease.2 A meaningful host parameter that can be measured in a quantitative and reproducible manner should provide an even earlier look at tissue destruction. 1

Amongst the host enzymes in GCF, alkaline phosphatase (ALP) was one of the first to be identified. It is a membrane bound glycoprotein produced by many cells within the area of periodontium and gingival crevice. It is released from polymorphonuclear neutrophils during inflammation, osteoblasts during bone formation, periodontal ligament fibroblasts during periodontal regeneration. Thus, it has dual involvement in the process of periodontal inflammation and healing / regeneration3. Alkaline phosphatase is stored in specific granules and secretory vesicles of neutrophils and is mainly released during their migration to the site of infection. It allows bone mineralization by releasing an organic phosphate and by hydrolysing inorganic pyrophosphate.4 ALP activity is valuable to clinicians because enzymatic modifications occur at the GCF level earlier than clinically evident modifications.

The aim of this study was to assess the total activity of alkaline

phosphatase in GCF collected from healthy sites, sites with gingivitis and chronic adult periodontitis, and to find its correlation with clinical parameters.

Materials and method

Subject and site selection

A total of 18 subjects (8 females and 10 males) in the age group of 20 to 55 years were selected. The subjects selected for the study were categorized into 3 groups

Group I: consists of 6 healthy subjects with clinically healthy periodontium (probing pocket depth not exceeding 3mm, no bleeding on probing and no evidence of bone loss).

Group II: consists of 6 patients with gingivitis (probing pocket depth not exceeding 4mm, presence of bleeding on probing and no evidence of bone loss).

Group III: consists of 6 patients with chronic adult periodontitis (pocket depth exceeding 4mm, presence of bleeding on probing and radiographic evidence of bone loss).

Patients who were suffering from systemic diseases and were on systemic antimicrobial and/or anti – inflammatory drug therapy within 3 months prior to this study or who had any periodontal treatment within 6 months prior to this study and who had teeth with a fixed or removable prosthesis were excluded.

GCF sample collection was done from 3 randomly selected sites of each patient in each group.

Clinical parameters:-

- Plaque index (P.I) : Silness and Loe (1964)
- Gingival index (G.I) : Loe and Silness (1963)
- Sulcus bleeding index (S.B.I) : Muhlemann and Son
- Probing pocket depth (P.P.D) : Measured with William's calibrated periodontal probe

Method of study

Prior to the crevicular fluid collection, supragingival plaque was scored using plaque index. After supragingival plaque was removed from each tooth with cotton pellets, the individual tooth site was gently air-dried and isolated with cotton rolls.

GCF samples were collected with calibrated capillary tubes and the sampling time for each site was 30 seconds. GCF was transferred with a jet of air pressure from capillary tube into eppendorf tube containing 250µl of normal saline and stored at -20 ° C until it was analysed with semiautomated analyser. Gingival inflammation was scored following crevicular fluid collection using gingival index (G.I). Probing depth measures were obtained from the sample sites using a William's periodontal probe (Hu-Friedy, IL, USA).

Enzyme Assay

ALP levels were determined by using an ALP kit by Accurex biomedical pvt. Ltd. and sample was assayed according to the kit's instructions. When samples were added to ALP kit, alkaline phosphatase in the sample hydrolyses para-nitrophenyl phosphate into para nitrophenol and phosphate, in the presence of magnesium ions. Under alkaline conditions a colourless p-nitrophenol is converted to 4-nitrophenoxide which develops a very intense yellow colour. The rate of increase of absorbance of the reaction mixture at 405nm due to liberation of paranitrophenol is proportional to the alkaline phosphatase activity. The spectrophotometric analysis was done by using semiautoanalyser.

Statistical analysis

Data were expressed as means and standard deviations. Data analysis was performed using SPSS as software for statistics. For comparison between the groups, ANOVA test including Bonferrini adjusted Wilcoxon signed rank test was used. In addition, Pearson's rank correlation coefficient was used in order to examine the relationship between clinical and biochemical parameters. The null hypothesis was rejected at p – value < 0.05

Results

- In group I, low levels of ALP activity were found. (Table 1)
- Increased GCF ALP activity in group II (gingivitis) patients as compared to healthy sites but this was not found to be statistically significant. (Table 1)
- GCF ALP activity was significantly increased in chronic adult periodontitis as compared to that in gingivitis. (Table 2)
- Relationship between P.I, G.I, B.I of patients with chronic adult periodontitis and ALP activity have failed to show a significant correlation (Table 3)
- GCF ALP activity was found to be positively correlated to probing depth. (Table 3)

Table 1: Comparison of plaque index, gingival index, bleeding index, probing pocket depth, Alkaline phosphatase total activity between Group I and Group II.

Parameter	Group I Mean S.D	Group II Mean S.D	p - Value	Significance
Plaque Index	0	1.51 0.59	0.001	S
Gingival Index	0	1.81 0.61	0.001	S
Bleeding Index	0	1.87 0.88	0.001	S
Probing pocket depth	1.06 0.12	2.57 0.37	0.001	S
Total ALP activity(µU/2 sites)	4264.58 724.06	5797.92 855.43	0.301	NS

ALP: Alkaline phosphatase activity; S: Significant; NS: Non significant

Table 2: Comparison of plaque index, gingival index, bleeding index, probing depth, Total ALP activity between Group II and III.

Parameter	Group II Mean SD	Group III Mean SD	p-Value	Significance
Plaque index	1.51 0.59	2.09 0.63	0.196	NS
Gingival index	1.81 0.61	2.17 0.12	0.321	NS
Bleeding score	1.87 0.88	2.61 0.53	0.145	NS
Probing pocket depth	2.57 0.37	3.79 0.39	0.001	S
Total ALP activity(µU/2 sites)	5797.92 855.43	8473.33 2377.01	0.024	S

NS- Non significant; S- Statistically Significant

Table 3: Correlation of Total ALP activity with plaque index, gingival index, bleeding index, probing depth in Group II and III.

Variable	Group II ALP activity Significance	Group III ALP activity Significance
Plaque index	0.911 NS	0.895 NS
Gingival index	0.912 NS	-0.958 NS
Bleeding index	-0.256 NS	0.159 NS
Probing pocket depth	0.488 NS	0.011 S

NS- Non significant ; S- Statistically Significant

Discussion

The processes responsible for the destruction of the human periodontium are highly complex and the vast range of biological substances is involved5.One area that is receiving a great deal of attention is the biochemical analysis of gingival crevicular fluid6

for diagnosing inflammation of periodontal tissues.

The potential of alkaline phosphatase as an important biochemical marker of GCF was identified by Ishikawa and Cimasoni7 in 1970. They quantified ALP in GCF using the colorimetric PNPP method and demonstrated levels of enzyme in GCF are three times greater than in serum. They also showed a significant correlation between ALP concentration in GCF and pocket depth which is in agreement to our study. This indicates periodontal bone also as a possible source of enzyme. It is indeed known that ALP is highly active in this tissue8.

Since GCF volume is influenced by many other factors such as flow rate, gingival trauma, and repeat sampling, it is logical to expect that total enzyme levels in GCF will correlate better with disease than enzyme concentrations6.

A significant correlation between gingival index and alkaline phosphatase could not be established in our study in contrast to the study of Chapple et al 1994 which reports that as the gingival index values increased, total ALP increased and this was significant in the early stages of gingivitis.(9)

In healthy gingiva, low levels of ALP activity were found. Increased GCF ALP activity in gingivitis cases as compared to that in healthy gingiva but this was not found to be statistically significant.

GCF ALP activity was found to be positively correlated to probing depth. This finding is in association with the findings of Ishikawa and Cimasoni7. GCF ALP activity was significantly increased in chronic adult periodontitis. An increase of ALP activity has been shown by histochemical methods in inflamed gingiva, both in humans and in ferrets7 .There are still several questions that remain to be answered, such as the exact source of the alkaline phosphatase and the nature of the isoenzyme(s) being measured. ALP is produced by many cells in the periodontal environment, the principal sources being PMNL, bacterial sources within supragingival and subgingival plaque, and osteoblast and fibroblast activity, with a small contribution from serum5.There is neutrophil predominance in the pocket epithelium and pocket itself and the major source of ALP during inflammation has been proposed to be neutrophils. This postulate is further strengthened by the results of the plaque assays and levamisole inhibition studies, which suggested that bacterial ALP contributed < 20% to total GCF ALP activity.9

It seems logical that all three sources would be raised in periodontal disease. If the source of GCF ALP is primarily the PMNL , it would form a potentially powerful marker of inflammation. However if the primary source is that of host connective tissue, then the assay may be useful in monitoring tissue healing as well as destructive processes5.

GCF collection and analysis are not without problems. The amount of fluid available per site is generally below a microlitre in volume and the elution of potential biochemical markers from the sample filters used is often difficult. Therefore we preferred precalibrated capillary tubes to collect pooled samples of GCF from randomly selected sites. Saliva contamination is a potential problem when sampling GCF and this could clearly influence results expressed as ALP concentration. ALP levels in saliva are extremely low and unlikely to influence results for GCF ALP expressed as total enzyme in a 30 – second sample.6

Conclusion

Alkaline phosphatase can be considered as a periodontal disease marker as it can distinguish between healthy and inflamed sites. Total activity of ALP was significantly higher in periodontitis as compared to gingivitis sites, which in turn showed levels of ALP higher than those found in healthy sites. It was found to be significantly and positively correlated with probing depth.

Alkaline phosphatase activity is valuable to clinicians because it can quantitatively evaluate the inflammatory status of gingival and periodontal tissues. Thus it can be used as an adjunct to clinical indices of inflammation . However in order to better define the potential of this mediator for diagnosis, additional longitudinal studies are needed.

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