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Evaluation of Hydrolytic Enzymes among Smokers with Chronic Periodontitis.

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ABSTRACT

Background: This study was done to evaluate the level of hydrolytic enzymes ALP and ACP in GCFamong chronic periodontitis patients with smoking habit. **Methods**: A total of 80 subjects were selected for the study. The study population was further divided into 4 group (Group 1 – clinically healthy periodontium, group – 2 gingivitis, group – 3 periodontitis, group - 4 smokers with periodontitis). Based on clinical assessment of probing depth , bleeding on probing and radiographic evaluation of alveolar bone loss, GCF samples were taken to assess the level of enzymes. **Results**: Obtained results shows statistically significant increase in the level of ALP activity in GCF from periodontitis patients with smoking habit. There was positive correlation between the activity of examined GCF enzymes and values of the plaque index, gingival index and periodontal disease index. **Conclusion**: Based on these results, it can be assume that ALP activity in GCF may be used as potent biochemical markers for periodontal destruction.

Keywords: Acid phosphatase, Alkaline phosphatase, Periodontitis, Smokers.

INTRODUCTION

The diagnosis of periodontal diseases have been determined traditionally on the basis of clinical evaluation comprising the presence of microbial deposits, extent of inflammation, probing pocket depth (PD), attachment loss, and radiographic assessment of alveolar bone loss.[1] However, the conventional methods for periodontal disease diagnosis have a few limitations associated in terms of accuracy, the ability to predict ongoing or future disease activity, and ability to reflect even marked histological changes.[2,3] Advances in oral and periodontal disease diagnostic research are moving toward many new methodologies for early detection of active disease, predict future disease progression and evaluate the response to periodontal therapy, thereby improving the clinical management of periodontal patients which will eventually result in expedited treatment. These include genetic susceptibility analysis, microbiologic analysis, and biochemical analysis.

Periodontal risk can be identified and quantified by objective measures such as biomarkers. Biomarkers of disease in succession play an important role in life sciences and have begun to assume a greater role in diagnosis, monitoring the therapy outcomes and drug discovery. Oral fluid biomarkers that have been studied for periodontal diagnosis include proteins of host origin (e.g., enzymes and immunoglobulins), phenotypic markers, host cells (e.g., PMNs), hormones, bacteria and bacterial products, ions, and volatile compounds. Several enzymes that are evaluated for the early diagnosis of periodontal disease are aspartate and alanine aminotransferase (AST, ALT), lactate dehydrogenase (LDH), creatine kinase (CK), alkaline and acid phosphatase (ALP, ACP), and gamma glutamiltransferase (GGT).[4-6]

ALP is a catalyzing enzyme that accelerates the removal of phosphate groups in the 5 and 3 positions from a variety of molecules, including nucleotides, proteins, and alkaloids. Although present in all tissues, ALP is particularly concentrated in the bone, liver, bile duct, kidney and placenta. It is a membrane-bound glycoprotein produced by various number of cells such as polymorphonuclear leukocytes, macrophages, fibroblasts and osteoblasts, within the area of the periodontium and gingival crevice.[7] Of interest in oral health is the association between ALP and periodontal disease.

Acid phosphatase, a lysosomal enzyme catalyses the hydrolysis of orthophosphoric monoester to an alcohol and orthophosphate. Zinc and magnesium are its cofactors. It is present in high concentrations in the prostate gland, and is also present in red

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cells, platelets, bone, liver, and spleen. Acid phosphatase are also found in neutrophils,[8] osteoclast,[9,10] and in membrane-coating granules present in keratinized epithelia.[11] They are also produced by plaque bacteria.

Risk elements that play an important role in disease progression are catogarised in risk factors, risk markers, risk determinant and risk indicators. "Risk factors" are characteristics of the person or environment that, when present, directly result in an increased likelihood that a person will get a disease and, when absent, directly result in a decreased likelihood of disease.[12]

Smoking has been implicated as a strong risk factor for periodontitis which contributes to the etiology of the most severe cases of periodontal disease. A direct causal relationship between smoking exposure and the prevalence and severity of periodontal disease has been firmly established.[13,14] According to the National Health and Nutrition Examination Survey III, smokers were four times as likely to have periodontitis as persons who had never smoked after adjusting for age, gender, race/ethnicity, education, and income/poverty ratio. Therefore the present study had been designed to evaluate the correlation of ALP and ACP activity in GCF among smokers with chronic periodontitis.

MATERIALS AND METHODS

Examination included 80 male patients, aged 20 – 40 years. The study population was further divided into 20 patients with gingivitis, 20 patients with periodontitis, 20 patients with periodontitis and smoking habit and 20 healthy adult volunteers. All subjects had good general health with no history of systemic disease. Alcoholic, pan chewers, drug abuse , patients who had periodontal therapy done 6 months prior to the study, patients under any systemic antibiotic and or anti-inflammatory drug therapy within 3 months prior to this study were excluded from this study. Patients with lesser than 20 permanent teeth and teeth with fixed or removal prosthetics were also excluded. As the initial examination, each subject completed a detailed medical questionnaire and received a complete periodontal examination, which included: Plaque index (PI), Gingival index (GI), Periodontal disease index (PDI), bleeding on probing (BOP) and probing depth (PD). Clinical periodontal recordings were performed at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) on each tooth using UNC – 15 probe. The study protocol was explained to the participants and written inform consent was obtained.

The study population was divided into:

- Group 1 - 20 subjects with clinically healthy periodontium with no bleeding on probing, no radiographic evidence of alveolar bone loss and greater than 90% of the sites exhibiting PD not exceeding 3mm.
- Group 2 – 20 subjects with gingivitis with PD not exceeding 3mm, presence of bleeding on probing and no radiographic evidence of alveolar bone loss.
- Group 3 – 20 subjects with periodontitis with PD exceeding greater than 5 mm, presence of bleeding on probing and presence of radiographic evidence of alveolar bone loss.
- Group 4 ¬– 20 subjects with periodontitis and smoking habit with PD exceeding greater than 5 mm, presence of bleeding on probing and presence of radiographic evidence of alveolar bone loss.

Gingival crevicular fluid sampling:

Gingival crevicular fluid samples for evaluating acid and alkaline phosphatase was collected from the same site with greatest probing depth after isolation and preparation of the concerned tooth using calibrated microcapillary tubes as per the recommendation of cimasoni et al 1969.[15] The sampling time was 30 seconds. GCF samples were then transferred with a jet pressure from the capillary tube into eppendorf tube containing 200 µl of normal saline (prepared by using 0.85 gms of NaCl and in 100 ml of distal water). A total of 60 GCF samples collected were stored at - 80 °C until it was analyzed using spectrophotometer.

Determination of alkaline and acid phosphatase activity:

ALP and ACP activity was measured by spectrophotometer using an ALP and ACP determination kit (CORAL DIAGNOSIS) According to the manufacturer's instructions.

Determination of alkaline phosphatase activity:

After homogenizing the mixture, working reagent was added to the distal water in the blank, stand and controls test tubes and incubated for 37°C for 3 minutes. After which the phenol standard was added to the stand and then the samples were added to the test tubes and incubated at 37 °C for 15 mins. After 15 mins colour reagent was added and the absorbance was red using spectrophotometer. Intensity of the colour formed is directly proportional to activity of ALP present in the sample.

Total ALP activity in each group was estimated using the formula:

$$\text{Total ALP activity in K.A units} = \frac{\text{AbsT} - \text{AbsC}}{\text{AbsT} - \text{AbsC}} \times 10.$$

Determination of acid phosphatase activity:

After homogenizing the mixture, working reagent was added to the distal water in the blank, stand

and controls test tubes and incubated for 37°C for 3 minutes. After which the phenol standard was added to the stand and then the samples were added to the test tubes and incubated at 37 °C for 60 mins. After 60 mins colour reagent was added and the absorbance was red using spectrophotometer. Intensity of the colour formed is directly proportional to activity of ACP present in the sample.

Total ACP activity in each group was estimated using the formula:

Total ACP activity in K.A units

$$= \frac{AbsT - AbsC}{AbsT - AbsC} \times 5.$$

Statistical analysis:

Data was presented as mean and standard deviations. Data analysis was performed by using SPSS as software for statistics. For comparison between groups Post Hoc ANOVA test was used. Probabilities of less than 0.05 were accepted as significant.

RESULTS

The results of the present study shows that GCF ALP and ACP activity was higher in patients with gingivitis , periodontitis and smokers with periodontitis when compared with the control. The ALP activity was highest in smokers with periodontitis when campared to the other 3 groups.

Table 1: Comparison between experimental groups and study variable.

Groups	Number	Particulars	ALP activity (mean values)	ACP activity (mean values)
Group – 1 clinically healthy periodontium.	20	Mean	5.89	1.48
Group – 2 Gingivitis	20	Mean	12.1	3.68
Group – 3 Periodontitis	20	Mean	13.68	3.34
Group – 4 Smokers with Periodontitis	20	Mean	29.39	3.68

Table 2: Correlation between plaque index with ALP and ACP

Groups	Number	Particulars	Plaque index	ALP (K.A. units)	ACP (K.A. units)	ALP activity (K.A.UNITS) significance	ACP activity (K.A.UNITS) significance.
Group – 2 Gingivitis	20	Mean	1.9900	12.10	3.68	0.034 (S)	0.320 (NS)
Group – 3 Periodontitis	20	Mean	2.3260	13.68	3.34	0.037 (S)	0.314 (NS)
Group – 4 Smokers with Periodontitis	20	Mean	2.2460	29.39	3.68	0.000 (S)	0.258 (NS)

p - Value < 0.05 statistically significant

Table 3: Correlation between gingival index with ALP and ACP

Groups	Number	Particulars	Gingival Bleeding Index	ALP (K.A. units)	ACP (K.A. units)	ALP activity (K.A.UNITS) significance	ACP activity (K.A.UNITS) significance
Group – 2 Gingivitis	20	Mean	2.0380	12.10	3.68	0.035 (S)	0 .333 (NS)
Group – 3 Periodontitis	20	Mean	2.3260	13.68	3.34	0.037 (S)	0.314 (NS)
Group – 4Smokers with Periodontitis	20	Mean	2.3180	29.39	3.68	0.000 (S)	0.268 (NS)

p - Value < 0.05 statistically significant

Table 4: Correlation between periodontal disease index with ALP and ACP

Groups	Number	Particulars	Periodontal Disease Index	ALP (K.A. units)	ACP (K.A. units)	ALP activity (K.A.UNITS) significance	ACP activity (K.A.UNITS) significance
Group – 2 Gingivitis	20	Mean	2.9880	12.10	3.68	0.036 (S)	0 .332 (NS)
Group – 3 Periodontitis	20	Mean	5.0120	13.68	3.34	0.039 (S)	0.310 (NS)
Group – 4 Smokers with Periodontitis	20	Mean	4.5320	29.39	3.68	0.000 (S)	0.278 (NS)

p - Value < 0.05 statistically significant

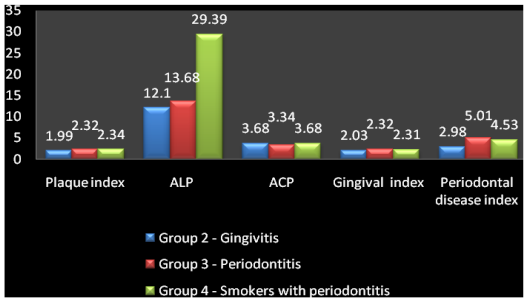

Figure 1: Bar chart which compares the ALP and ACP activity, Plaque and gingival bleeding index values among Group 2, Group 3 and Group 4.

DISCUSSION

Periodontitis is one of the major threats to oral as well as to overall health. The process involved in the destruction of the periodontium is highly complex and vast ranges of biological substances are involved. Among various risk factors, Cigarette smoking is arguably and the strongest behavioral risk factor for the incidence and progression of periodontitis.[16] This study was done to evaluate the level of hydrolytic enzymes ALP and ACP as potential biochemical GCF markers among chronic periodontitis patients with smoking habit.

ALP and ACP are intracellular enzymes present in most of the tissues, and organs particularly in bones. The mean ALP levels of Group 1(clinically healthy periodontium) was found to be lower than other three groups (Gingivitis, periodontitis and in smokers with periodontitis). In support of this finding, Lilja E et al et al 1984,[17] have shown through histochemical techniques that ALP is present in the normal periodontal membrane in rats. Lindhe J et al 2008,[18] also stated that certain leukocytes can be seen in junctional epithelium of healthy gingiva . Gao J et al et al 1999,[19] stated that ALP is essential enzyme, and it is a part of normal turnover of periodontal ligament, root cementum and bone hemostatis. This supports the presence of ALP and ACP even in clinically healthy periodontium in the present study.

The results of the present study shows that the level of ALP and ACP are significantly elevated in group 2 (12.10 K.A units, 3.68 K.A units) group 3 (13.68 K.A units, 3.34 K.A units) and group 4 (29.39 K.A units, 3.68 K.A units) when compared to the clinically healthy subjects (group 1) which signifies the value of ALP and ACP as a markers of periodontal inflammation. These enzymes are indicators of higher levels of cellular damage and their increased activities in GCF are a consequence of their increased release from the damaged cells of the soft tissues of the periodontium and are a reflection of metabolic changes in inflamed gingiva.[19,20]

[Table 2-4] shows a positive correlation between the ALP and ACP activity and values of the plaque index, gingival index and periodontal disease index which is in concordance with the study done by Todorovic et al 2006.[20] [Table 1] shows that periodontitis patients with smoking habit had significantly highest level of ALP activity which is in agreement with the studies conducted by Sarita Dabra et al 2012.[22]

The possible explanation for the increase in the level of ALP in group 2 and group 3 is that during the progression of the periodontal disease, enzymes are released from the PMNLs, inflammatory, epithelial and connective tissue cells and also from the dead and the dying cells of the periodontium of the affected sites.[7] As PMNLs are the major source of ALP, they could have contributed to the increased levels of ALP in GCF through secondary granules release.[2] Microorganisms like Prevotella intermedia and streptococcus sanguis also shows higher ALP activity. Thus increase in activity of ALP in group 2 (gingivitis) could be due to increase in the number of PNMLs and bacteria in the gingival sulcus.

Periodontal pockets are chronic inflammatory lesions and are constantly undergoing repair. Complete repair does not occur because of persistent irritant and these irritant continues to stimulate fluid and cellular exudates which in turn causes degeneration of new tissue elements. Apart from increase in the number of PMNLs and bacterial activity in periodontitis, one of the major mechanism of collagen loss is that fibroblast phagocytise collagen fibres. Increase in fibroblast activity contributes to the total ALP level.[7,23] This could be the reason for the increase in level of ALP activity in group 3.

Among the three groups the mean value of ALP is highest in the smokers with periodontitis group (group 4). The possible explanation could be that smoke exerted a direct irritating effect on the gingiva giving rise to gingivitis and that nicotine could cause contraction of the capillaries, thus interfering with the nutrition of the gingiva which consequently became less resistant to infection.[24] Smoking-induced chronic hypoxia of periodontal tissues causes greater severity of periodontal disease seen in smokers by quantitative increase in the level of anaerobes.[25] Trikilis et al 1999 found that subgingival temperatures are lower in smokers than nonsmokers. The decreased subgingival temperature in smokers might reflect the reduced activity of periodontal cell.[26]

Smokers also exhibit elevated neutrophil-mediated destruction which may lead to increased secretion of potentially tissue-destructive products. In addition, nicotine may prolong the lifespan of neutrophils in tissue by delaying the process of programmed cell death (apoptosis), 27 which contributes to the total increase ALP level in group 4. Among the various periodontopathogenic bacteria like Prevotella intermedia and

Porphyromaonas gingivalis are known to have high ALP activity and in this study group 4 also showned a positive correlation between plaque index value and ALP level which is in concordance with the study done by Ellis E et al 2007.[28]

Chronic periodontitis is also associated with elevated levels of systemic inflammatory markers and production of several lysosomal enzymes. Acid phosphatase is an intracellular enzyme associated with bone metabolism. It is present in neutrophils and considered a lysosomal marker.Clinical and microbiological studies have identified increased activity of ACP might be a consequence of destructive processes in alveolar bone and associated with advance stage of periodontal disease. In the present study Acid phosphatase activity had non- significant difference between smokers with periodontitis, periodontitis and gingivitis patients which is in contrast with the study done by Pushparani DS et al 2015,[29] where the level of ACP enzyme in the gingival tissue correlated with the severity of periodontitis.

CONCLUSION

Within the limitation of this study it can be concluded that GCF level of alkaline phosphatase was significantly higher in patients with gingivitis and periodontitis and highest in periodontits group. Thus ALP can be considered as a potential biomarker for the detection and progression of periodontal disease whereas the evaluation of acid phosphatase does not seem to have any significant diagnostic value.

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