Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr ICV 2018= 82.06

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

Original Research

Evaluation of Alkaline Phosphatase in Gingival Crevicular Fluid among Chronic Periodontitis Patients with Smoking Habit

K. Hameed Fathima[1], V.S.Harish.[2]

[1,2]Assistant Professor, Department of Dentistry, Sri Muthukumar Medical College hospital and Research Institute, Chennai India,

***ABSTRACT*:**

Background: This study was done to evaluate the level of alkaline phosphatase as potential biochemical gingival crevicular fluid marker among chronic periodontitis patients with smoking habit. **Materials and 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 and Group - 4 smokers with periodontitis) .Based on clinical assessment of probing depth , bleeding on probing and radiographic evaluation of alveolar bone loss , gingival crevicular fluid samples were taken to assess the level of enzymes . **Results**: Obtained results shows statistically significant increase in the level of alkaline phosphatase activity in gingival crevicular fluid from periodontitis patients with smoking habit . There was positive correlation between the activity of examined gingival crevicular fluid enzymes and values of the plaque index , gingival index and periodontal disease index. **Conclusion**: Based on these results, it can be assume that alkaline phosphatase activity in gingival crevicular fluid may be used as potent biochemical markers for periodontal destruction.

Key words: Alkaline phosphatase, Gingival crevicular fluid, Periodontitis , Smokers.

Received: 13 March, 2019 Revised: 22 April 2019 Accepted: 25 April 2019

Corresponding author: Dr. K. Hameed Fathima, Assistant Professor, Department of Dentistry, Sri Muthukumar Medical College hospital and Research Institute, Chennai India

This article may be cited as: Fathima KH, Harish VS Evaluation of Alkaline Phosphatase in Gingival Crevicular Fluid among Chronic Periodontitis Patients with Smoking Habit. J Adv Med Dent Scie Res 2019;7(7): 163-167.

INTRODUCTION:

Periodontal disease is the result of a complex interplay among bacterial challenge, host response, and other modifying factors. Clinical measurements used in diagnosis of periodontal diseases are often of limited usefulness, in that they are indications of previous periodontal disease rather than the present disease activity. Biochemical mediators in oral fluids like saliva and gingival crevicular fluid (GCF) are highly beneficial in the determination of current periodontal status. These substances are known as biomarkers. They help in determination of inflammatory mediator levels, as they are good indicators of inflammatory activity.[1] Biomarkers, whether produced by normal healthy individuals or by individuals affected by specific systemic diseases are telltale molecules that could be used to monitor health status, disease onset, treatment response and outcome. Informative biomarkers can further serve as early sentinels of disease.[2] Although a single specific target biomarker for periodontal disease has not been identified, combinations of putative biomarkers of disease have been evaluated in oral fluids and demonstrated significant potential as panels of targets for the development of an oral fluid fingerprint of periodontal disease status.

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.[3-7]Among these enzymes of host origin are further divided into proteolytic and hydrolytic enzymes. Intracellular enzymes are increasingly released from the damaged cells of periodontal tissues into the GCF and saliva. 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).[8-10]

Alkaline phosphatase (ALP) is a hydrolase enzyme, a membrane based glycoprotein produced by many cells within the area of the periodontium and gingival crevice.[11] It is released from polymorphonuclear neutrophils during inflammation, osteoblasts during bone formation, and periodontal ligament fibroblasts during periodontal regeneration.[12] Since increase in ALP in serum has been associated with bone disease, local elevations in GCF could also reflect local soft and hard tissue alterations in active periodontal disease.

Cigarette smoking an environmental risk factor that represents a major preventable cause of human disease .[13] Tobacco smoke contains over 3800 chemicals, including carbon monoxide, hydrogen cyanide, and reactive oxidizing radicals, and sixty of these chemicals are known or suspected to be carcinogens.[14] The use of tobacco products, in general, and smoking products, in particular, is the major preventable risk factor for the initiation and progression of periodontal diseases.[15-17] Smoking affects the levels of some well-known markers of gingival health.[18,19]Limited number of studies have evaluated the effect of smoking on the levels of ALP in GCF, which is a potentially powerful marker of periodontal disease activity. Thus the present study was done to evaluate the level of ALP as potential biochemical GCF markers among chronic periodontitis patients with smoking habit .

MATERIALS AND METHOD:

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 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.[20] 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 phosphatase activity:

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

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

The obtained 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 AND 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. [20] This study was

done to evaluate the level of hydrolytic enzymes ALP as potential biochemical GCF markers among chronic periodontitis patients with smoking habit.This comparative study was done among 80 subjects in the Department of Periodontology at S.R.M Dental college, Chennai. Systemically healthy male patients without any habits were included in this study in order to prevent the gender difference report as well as the influence of risk elements effect on ALP activity.

Among the host enzymes, the first one identified one was ALP. It is most effective in an alkaline environment. The optimal pH for the ctivity of ALP is 8.0-8.5 depending on the source .[12] ALP is stored in specific granules as well as secreted by vesicles of neutrophils and is mainly released during their migration to the site of infection. [21] Ishikawa and Cimasoni identified the pontential of ALP as an important biochemical marker of GCF.[22]

The results of the present study shows that the level of ALP are significantly elevated in group 2 (12.10 K.A units) group 3 (13.68 K.A units) group 4(29.39 K.A units) (when compared to the clinically healthy subjects (group 1) which signifies the value of ALP as a markers of periodontal inflammation.

Table 1 shows that periodontitis patients with smoking habit significantly higher level of ALP activity which was in agreement with the studies conducted by Erdemir EO et al 2006 [19]and Vishakha Grover et al 2016 . [23] Table 2, 3 ,4 and figure 1 shows a positive correlation between the ALP 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 [24]and Ellis E et al et al 2007. [25] 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. [26] As PMNLs are the major source of ALP, they could have contributed to the increased levels of ALP in GCF through secondary granules release. [27] Microorganisms like Prevotella intermedia and streptococcus sanguis also shows higher ALP activity . [28] 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.

Smoking creates an environment that favors colonization of pathogens in shallow sites and could help to explain the initiation of disease at new sites and the development of periodontitis in smokers.Reports have shown higher proportions or the prevalence of exogenous or commensal flora in moderate to deep probing depths in smokers that point toward an adverse effect of smoking on the host response.[29] Smoking impairs various aspects of the innate and adaptive host responses including alterations in neutrophil function, antibody production, fibroblast activities, vascular factors, and inflammatory mediator production. Smokers exhibit elevated total white blood cell and granulocyte counts in their systemic circulation. Polymorphonuclear leukocyte viability and functions such as phagocytosis, superoxide and hydrogen peroxide generation, integrin expression, and protease inhibitor production can be altered by cigarette smoking or various tobacco components. Neutrophils play a key role in both host protection and tissue destruction. Smoking appears to elicit the more destructive activities of polymorphonuclear leukocytes [29] this could be the reason for the increase in ALP activity in group 4 when compared to the other groups.

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. Smoking results in increased periodontal destruction by altering the host response through impairment of the normal host response in neutralizing infection and alteration results in destruction of the surrounding periodontal tissues.[30] Thus increased in the activity of ALP in group 4 is probably a consequence of pathological processes in periodontal tissues modified by risk factor as ALP is produced by PMNs, osteoblasts, macrophages, fibroblasts and plaque bacteria within periodontal tissue or periodontal pocket.[31] This could be the possible reason for the highest level of ALP activity in group 4 when compared to the other groups.

Table 1 : Comparison between experimental group and study variable

Groups	**Number**	**Particulars**	**ALP activity (mean values)**
Group – 1 clinically healthy periodontium	20	Mean	5.89
Group – 2 Gingivitis	20	Mean	12.1
Group – 3 Periodontitis	20	Mean	13.64
Group – 4 Smokers with Periodontitis	20	Mean	29.39

Table 2: Correlation between plaque index with ALP

Groups	Number	Particulars	Plaque index	ALP (K.A.units)	ALP activity (K.A.units) Significance.
Group – 2 Gingivitis	20	Mean	1.9900	12.10	0.034 (S)
Group – 3 Periodontitis	20	Mean	2.3260	13.68	0.037 (S)
Group – 4 Smokers with Periodontitis	20	Mean	2.2460	29.39	0.000 (S)

p - Value < 0.05 statistically significant

Table 3: Correlation between gingival bleeding index with ALP

Groups	Number	Particulars	Gingival Bleeding Index	ALP (K.A. units)	ALP activity (K.A.units) Significance.
Group – 2 Gingivitis	20	Mean	2.0380	12.10	0.035 (S)
Group – 3 Periodontitis	20	Mean	2.3260	13.68	0.037 (S)
Group – 4 Smokers with Periodontitis	20	Mean	2.3180	29.39	0.000 (S)

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)	ALP activity (K.A.UNITS) Significance.
Group – 2 Gingivitis	20	Mean	2.9880	12.10	0.036 (S)
Group – 3 Periodontitis	20	Mean	5.0120	13.68	0.039 (S)
Group – 4 Smokers with Periodontitis	20	Mean	4.5320	29.39	0.000 (S)

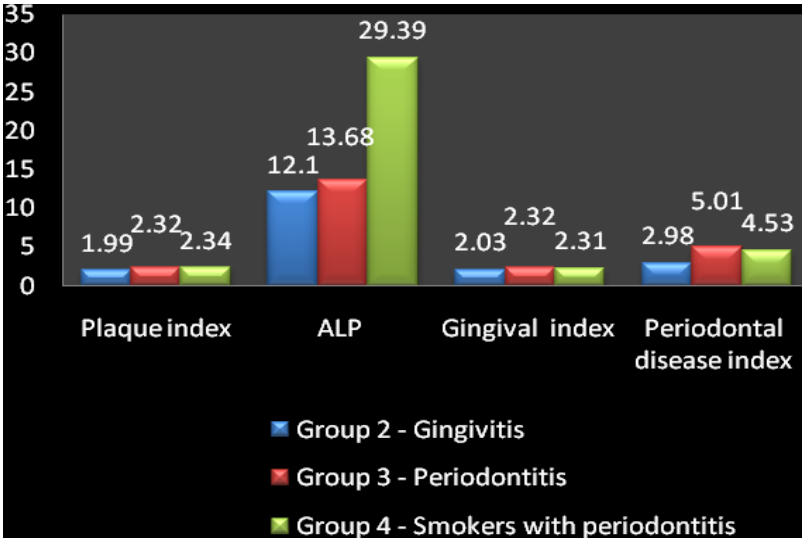

Figure 1: Bar chart which compares the ALP activity, Plaque index , Gingival index, Periodontal disease index values among gingivitis , periodontitis and smokers with periodontitis groups .

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 smokers with periodontits group. Thus ALP can be considered as a potential biomarker for the detection and progression of periodontal disease .

REFERENCES:

1. Taba M et al. Diagnostic biomarkers for oral and periodontal diseases. Dent Clin N Am. 2005; 49:551-71.
2. Colburn WA, 2003. Biomarkers in drug discovery and development: from target identification through drug marketing .Journal of Clinical Pharmacology;43(4):329-41.
3. Lamster IB, Grbic JT. Diagnosis of periodontal disease based on analysis of the host response. Periodontol 2000;7:83 -99.
4. Mandel ID.The diagnostic uses of saliva. J. Oral Pathol. Med 1990;19:119–125.
5. Ferguson DB.Current diagnostic uses of saliva. J. Dent. Res 1987;66:420–424.
6. Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis a review. J. Clin. Periodontol 2000;27:453–465.
7. Nakamura M, Slots J. Salivary enzymes: Origin and relationship to periodontal disease. J. Periodontal Res 1983;18:559–569.
8. Numabe Y, Hisano A, Kamoi K, Yoshie H, Ito K, Kurihara H. Analysis of saliva for periodontal diagnosis and monitoring. Periodontology 2004;40:115-9.
9. Ozmeric N. Advances in periodontal disease markers. Clin Chim Acta 2004;343:1-16.
10. McCulloch CA. Host enzymes in gingival crevicular fluid as diagnostic indicators of periodontitis. JClinPeriodontol.1994;21:497-506.
11. Bezerra AA et al. Evaluation of organic and inorganic components in saliva of patients with chronic periodontal disease. Rev Odonto Cienc . 2010;25:234-5.
12. Perinetti G, Paolantonio M, Femminella B, Serra E, Spoto G. Gingival crevicular fluid alkaline phosphatase activity reflects periodontal healing/recurrent inflammation phases in chronic periodontitis patients. J Periodontol. 2008;79:1200–7.
13. Tonetti MS. Cigarette smoking and periodontal diseases: Etiology and management of disease. Ann Periodontol 1998;3:88-101.
14. Eriksen MP, LeMaistre CA, Newell GR. Health hazards of passive smoking. Annu Rev Public Health 1988;9:47-70.
15. Genco RJ, Borgnakke WS. Risk factors for periodontal disease. Periodontol 2000 2013;62:59-94.
16. Johnson GK, Guthmiller JM. The impact of cigarette smoking on periodontal disease and treatment. Periodontol 2000 2007;44:178-94.
17. Nociti FH Jr., Casati MZ, Duarte PM. Current perspective of the impact of smoking on the progression and treatment of periodontitis. Periodontol 2000 2015;67:187-210.
18. Rivera-Hidalgo F. Smoking and periodontal disease. Periodontol 2000. 2003;32:50–8.
19. Erdemir EO, Duran I, Haliloglu S. The enzyme activity of alkaline phosphatase in gingival crevicular fluid of smokers and nonsmokers with chronic periodontitis. Cilt. 2006;30:25-32.
20. Cimasoni et al . Collection of gingival crevicular fluid for quantitative analysis . Journal of Dental Research 1969;48;159.
21. Daltabano et al. gingival crevicular fluid alkaline phosphatase levels in postmenopausal women: effect of phase I periodontal treatment. J Periodontal 2006;7:453-65
22. Ishikawa et al. alkaline phosphatase in human gingival fluid and its relation to periodontitis. Arch Oral Biol 1970;15:1401-4.
23. Vishakha Grover et al et al Correlation of alkaline phosphatase activity to clinical parameters of inflammation in smokers suffering from chronic periodontitis. J Indian Soc Periodontol. 2016 May-Jun; 20(3): 254–259.
24. Todorovic T et al. Salivary enzymes and periodontal disease. Med. Oral Patol. Oral Cir. Bucal.2006;11:115-9.
25. Ellis E et al. The role of alkaline phosphatase in mineralization. Curr Opin Orthop 2007;18:444-8.
26. Chapple IL et al. Prediction and diagnosis of attachment loss by enhanced chemiluminescent assay of cervicular fluid alkaline phosphates levels. J Clin Periodontol.1999;26:190-8.
27. Chapple IL et al . Site – specific alkaline phosphatase levels in gingival crevicular fluid in health and gingivitis: Cross-sectional studies. J Clin Periodontol. 1994;21: 409-14.
28. Shibita Y et al. Effective method for discriminating between oral bacterial and human alkaline phosphatase activity. Oral Microbiol Immunol.1994;9:35-9.
29. Johnson GK, Guthmiller JM. The impact of cigarette smoking on periodontal disease and treatment. Periodontol 2000. 2007;44:178–94.
30. Wolf BH et al. multivariate analysis of oral hygiene data from a representive sample. J Clin Periodontal 2001;28:891-4.
31. Chapple E et al. analysis of saliva for periodontal diagnosis - a review. JClin Periodontal 2000;27:453-65.