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Comparative Evaluation of Alkaline Phosphatase (ALP) Levels in Gingival Crevicular Fluid (GCF) and Alveolar Bone Loss in Smokers and Non-Smokers with Chronic Periodontitis Patients: A Clinico-Biochemical Study

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ABSTRACT

Background: Chronic Periodontitis is a periodontal disease caused by dental plaque and is more prevalent and severe in smokers and is characterized by deeper pockets with greater attachment loss. The study was carried out to evaluate and compare the clinical parameters, Alkaline phosphatase levels in GCF and radiographical periodontal bone loss in smoker and non-smoker patients with chronic periodontitis.

Materials and Methods: 100 patients were recruited in study with 50 patients in each group (Group I and Group II). Ancillary parameters (Plaque index and Gingival Index) and clinical parameters (Probing depth and clinical attachment levels) were recorded in both the groups. GCF was collected with micropipettes and ALP levels were measured. RVG was taken in all the patients at four selected sites. Statistical analysis was done using independent t- test ($p \leq 0.05$).

Results: All ancillary parameters and clinical parameters were found to be statistically higher in smokers as compared to non-smokers ($p \leq 0.05$). GCF levels of ALP were found to be lower (753.395±49.418) in smokers than in non-smoker group (818.259±51.252).

Conclusion: In this study it was concluded that periodontal destruction is greater in smokers as compared to non- smokers with more alveolar bone loss and decreased alkaline Phosphatase levels in GCF of smokers.

Keywords: Gingival Crevicular fluid, Probing Depth, Alkaline Phosphatase, Smokers.

INTRODUCTION

Periodontal diseases are infections caused by dental plaque leading to progressive destruction of supporting tissues which gets modified by the alteration of host response to microbial aggression[1]. Various known risk factors that can affect this process are age, gender, socioeconomic status, genetic predisposition, certain systemic conditions and smoking. Out of all these factors use of tobacco mostly in the form of cigarette smoking, is considered as the most important environmental risk factor responsible for excess prevalence of periodontal disease in the population and has a direct influence on periodontal variables[2].

The role of cigarette smoking in the pathogenesis of periodontal disease has been extensively studied and well documented over the past two decades. The 1996 World Workshop in Periodontics reviewed a number of studies and confirmed that "smoking entailed an overall increased risk for severe periodontal disease and estimated overall odds ratio is 2.82[3]. Generally, assessment of risk

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shows that smoking is associated with 2 to 7 fold increase in risk for having periodontitis and/or periodontal tissue loss compared to non-smokers[4].

Earlier investigators had attributed the increased prevalence and severity of periodontal disease seen in smokers to the greater presence of plaque and calculus as compared to nonsmokers. However, with the better understanding of the host response, evidence suggests that the effect of smoking on periodontal status is independent from the plaque index and oral hygiene of individual. So, it can be considered that smoking is an independent environmental risk factor for periodontitis and it has a direct influence on periodontal tissues[5].

Chronic periodontitis is found to be more prevalent and more severe in smokers, characterized by deeper pockets and greater attachment loss, more pronounced radiographic evidence of furcation involvement, and increased alveolar bone loss. There is an established biologic rationale for the negative effect of smoking on periodontal tissues. It has an immunosuppressive effect on the host, adversely affecting host-bacterial interactions, and this alteration may be due to changes in the composition of subgingival plaque[5].

Smoking can lead to increased periodontal destruction by altering the host response through the impairment of the normal host response in neutralizing infection. It also provides a conducive environment for some of the periodontopathic organisms in the plaque considered as a risk factor for periodontal disease development[6]. Smoking exerts a strong, chronic, and dose-dependent suppressive effect on gingival bleeding on probing. This may be explained by the fact that one of the numerous tobacco smoke by-products, nicotine exerts local vasoconstriction, reduced blood flow, edema and inhibits early signs of periodontal problems by decreasing gingival inflammation, redness, and bleeding. The decreased gingival bleeding in smokers can also be attributed to the heavier keratinization of the gingiva in smokers. This would also induce a decreased local host response. So, smoking is thought mainly to affect the periodontal tissues by way of the vascular and immunological response of the body[7].

Clinical measurements used in diagnosis of periodontal diseases like probing depth, gingival recession, clinical attachment level using graduated periodontal probe provide limited information as they are indicators of previous periodontal disease rather than the present disease activity. 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 and biochemical analysis.

Biochemical methods in periodontal diagnosis have centred primarily on saliva, gingival crevicular fluid (GCF) and its constituents. The potential diagnostic importance of GCF was recognized more than 60 years ago[8]. Analysis of the GCF provides a non-invasive method of studying the host response factors in the periodontium during the initial diagnosis and treatment. It 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 GCF have been related to gingivitis, periodontitis and, most recently, to periodontal disease activity[9].

Periodontal disease leads to the destruction of periodontal tissues and the products of tissue destruction are seen in GCF. Intracellular enzymes are increasingly released from the damaged cells of periodontal tissues into the GCF and saliva. It has been shown that the levels of enzymes in GCF such as ß-glucuronidase (BG), elastase, collagenase, aspartate aminotransferase (AST) and alkaline phosphatase (ALP) have a possible correlation with periodontal destruction[10].

The host derived enzyme Alkaline phosphatase (orthophosphoric-monoester phosphohydrolase) plays a key role in bone metabolism. It is a membrane bound glycoprotein produced by many cells such as polymorphonuclear leukocytes, osteoblasts, macrophages and fibroblasts within the area of the periodontium and gingival crevice. It is detected in the parotid, submandibular, and minor salivary glands, as well as in desquamated epithelial cells, leukocytes, and bacteria from dental plaque. ALP is stored in specific granules and secretory vesicles of neutrophils and is mainly released during their migration to the site of infection[11].

The level of ALP is positively correlated with the severity of the periodontal disease and is one of the

potentially powerful markers of periodontal disease activity. ALP is considered to be an important indicator of bone formation and is a phenotypic marker for osteoblast cells. It allows bone mineralization by releasing an organic phosphate and by hydrolyzing inorganic pyrophosphate, a potent inhibitor of hydroxyapatite crystal formation and dissolution[12].

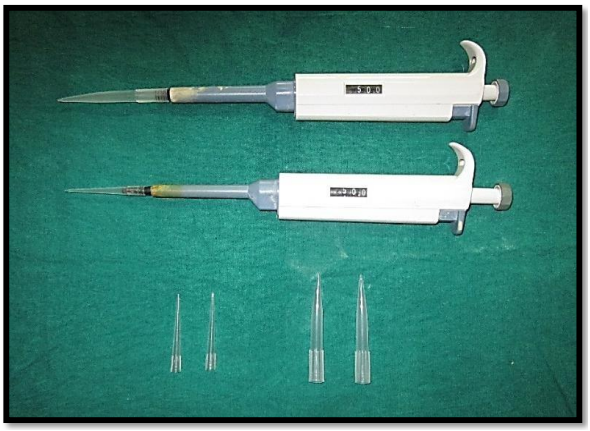

Fig 1: Micropipettes with disposable tips used for mixing reagent.

Fig 2: ERBA autoanalyzer for ALP levels evaluation.

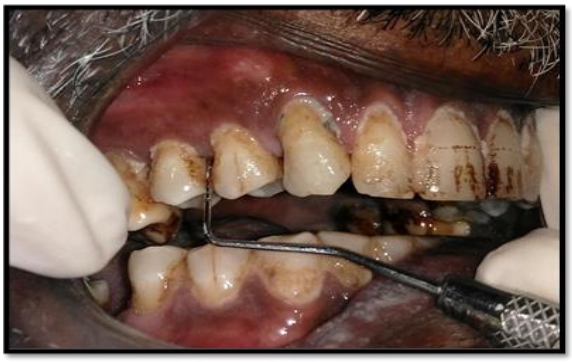

Fig 3: Probing depth in smokers.

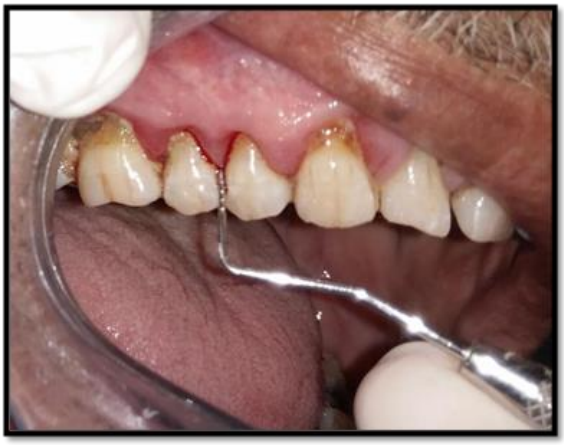

Fig 4: Probing depth in non-smokers.

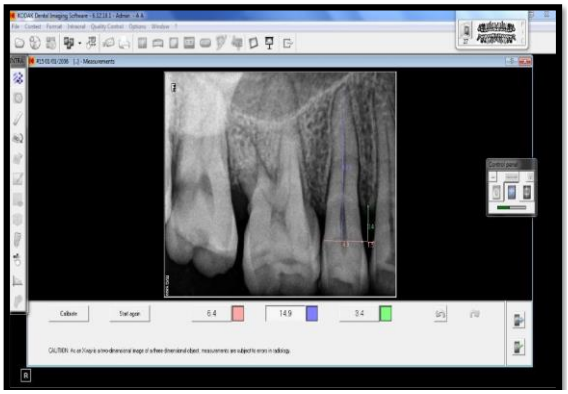

Fig 5: Calculation of radiographic alveolar bone loss using KODAK software.

Conventional periapical radiographs are used to detect bone loss in addition to clinical examination over the past few years. Systems that can generate radiographic digital images without the need for radiographic film have become available for use and has attracted a lot of attention in determining the depth, width, topography of bony defects and progression of the defect since loss of bone density and height should be evaluated using an automated instrument to diagnose periodontal lesions and assess the treatment success[13].

Alveolar bone is one of the tissues that is most affected by the progression of periodontal disease. A greater alveolar bone loss is seen among smokers on examining radiographically as compared to non-smokers. The reduction in bone height is 2.7 times greater in adult smokers than smokers. Smoking affects the mineral content of the bone tissue. Progressive loss of bone mineral with advancing age is even more prominent in smokers.

Thus, this study was conducted to evaluate and compare the clinical parameters, Alkaline phosphatase levels in GCF and radiographical periodontal bone loss in smoker and non-smoker patients with chronic periodontitis.

MATERIALS & METHODS

This cross-sectional, comparative, biochemical and radiographic study was carried out in 100 male patients, 50 smokers and 50 non-smokers in the age range of ***40-65*** years having chronic periodontitis, reporting to Department of Periodontology of Narsinhbhai Patel Dental College and Hospital, Visnagar. The subjects were examined under the inclusion and exclusion criteria and they were selected for study. Subjects who agreed to participate in the study were asked to sign (thumb impression in case of illiterate subjects) the written consent. The study was approved by the ethical committee of Narsinhbhai Patel Dental College and Hospital (NPDCH), Visnagar, before commencement of the study.

Patients who were included in the study had chronic periodontal disease with pocket depth of 5mm or more and/or clinical attachment loss of 1mm or more in around 30% of the existing natural teeth. Patients who were smoking 5 to 10 cigarettes per day for 5 years or more were recruited for the study. Patients who had taken a course of anti-inflammatory or anti-microbial therapy within the past 6 months and patients who underwent any surgical or nonsurgical periodontal therapy in the past 6 months were excluded from the study. Any systemic diseases and patients who were alcoholic, female patients, immunocompromised patients and former smokers were also excluded from the study.

Study Design:

The subjects were divided into 2 groups of 50 each:

Group I - Patients who had chronic periodontitis and met the smoking criteria.

Group II - Patients who had chronic periodontitis and were non-smokers.

Complete medical history and periodontal examination of the patients was done.

GCF collection was done with Hirschmann Micropipettes (SIGMA ALDRICH®) (Fig- 1). Supra gingival plaque and calculus were removed with hand instruments prior to the collection of GCF. Care was taken not to touch the gingival margin to prevent stimulation. The calibrated Hirschmann micropipette (0–5 µL range) were placed extracrevicularly at the mesiofacial, distofacial, or midfacial surface of the selected tooth. GCF was collected up to first marking of micropipette which corresponded to 1µl of GCF volume. GCF ALP assay was then done by ERBA ALP Kit (Fig 2). The sample collected in the micropipette was transferred into test tube with 100 µl normal saline for dilution. With the help of pipette 500µl of ALP reagent was mixed in the diluted 50µl GCF sample and shaked to get the uniform homogenous sample. The enzyme activity was recorded using an autoanalyzer and the result was obtained in the form of a digital readout.

Plaque Index[14], Gingival bleeding index[15], Probing pocket depth (Using William's calibrated probe) (Fig 3 and 4) and Clinical Attachment Level were recorded at baseline. All the data recordings were made by the same examiner, who was calibarated and standardized by another examiner.

Radiographic examination

The radiographic evaluation of the patient was done using Radiovisiography (RVG) (Fig-5). RVG was taken from 4 selected sites of the patient. Radiographs were obtained by the paralleling technique by the same operator and machine. A fixed anatomical point, i.e. **cementoenamel junction (CEJ)**, was considered for measurement. If the CEJ was destroyed by restorative treatment, the margin of the restoration was taken as landmark.

Statistical methods

The collected data was condensed and subjected to statistical analysis. Mean and standard deviation of all the parameters were calculated. Independent t-test was done to find significant difference between the means and level of significance was calculated. The p- value was set at ≤ 0.05The statistical analysis was done using SPSS version 23.

RESULTS

Table 1: Comparison of mean age between Smokers and Non – Smokers.

Group	N	Mean	Std. Deviation	Std. Error Mean	T value	P - value
Smokers	50	49.180	9.0276	1.2767	- 0.126	0.900
Non-smokers	50	49.400	8.4612	1.1966		

As shown in Table 1, the mean age in smokers group was 49.180±9.027 and in non-smokers group it was 49.40±8.461. There was no statistically significant difference in age between the smokers and non - smokers. The mean age was same in both groups.

Table 2: Comparison of Probing Depth between Smokers and Non – Smokers.

Group	N	Mean	Std. Deviation	Std. Error Mean	T value	P - value
Smokers	50	5.6060	.7213	0.0878	2.322	0.022*
Non-smokers	50	5.3338	.54910	.07765		

As shown in Table 2, mean probing depth in smoker group was 5.606±0.721 and in non-smokers group it was 5.333±0.549. There was statistically significant difference present between the probing depth score in smokers and non smokers. The mean levels of probing depth scores were higher in smokers when compared with that of non-smokers.

Table 3: Comparison of Clinical Attachment Level between Smokers and Non – Smokers.

Group	N	Mean	Std. Deviation	Std. Error Mean	T value	P - value
Smokers	50	4.7394	0.6212	0.1020	2.32	0.036*
Non-smokers	50	4.3678	.77252	.10925		

*-Statistically significant (P<0.05)

As shown in Table 3, mean clinical attachment level in smoker group was 4.734±0.621 and in non-smoker group it was 4.367±0.772. There was statistically significant difference present between the clinical attachment loss in smokers and non-smokers. The mean levels of Clinical attachment loss were higher in smokers when compared with that of non-smokers.

Table 4: Comparison of GCF Alkaline Phosphatase level between Smokers and Non –Smokers

Group	N	Mean	Std. Deviation	Std. Error Mean	T value	P - value
Smokers	50	753.3958	49.41833	6.98881	-6.447	<0.001**
Non-smokers	50	818.2592	51.25205	7.24813		

**statistically highly significant.

As shown in Table 4, mean GCF Alkaline phosphatase levels in smokers group was 753.395±49.418 and in non-smoker group it was 818.259±51.252. There was statistically highly significant difference present in the Biochemical analysis (ALP) scores between the smokers and non-smokers with higher values in the Non Smokers when compared with smokers with a p value of <0.01.

Table 5: Comparison of % of Radiographic Alveolar Bone Loss between Smokers and Non -Smokers.

Group	N	Mean	Std. Deviation	Std. Error Mean	T value	P - value
Smokers	50	32.4804%	4.58741%	.64876%	3.301	0.001**
Non-smokers	50	29.2082%	5.29866%	.74934%		

**- statistically highly significant.

As shown in Table 5, mean % of radiographic alveolar bone loss in smoker group was 32.480%±4.587% and in non-smoker group it was 29.208±5.298. There was statistically highly significant difference present in the mean % of bone loss (detected in radiographs) between the smokers and non-smokers with higher values in the Smokers when compared with non-smokers with a p value of <0.01.

DISCUSSION

Periodontitis is a multifactorial irreversible and cumulative condition, initiated and propagated by bacteria and host factors. It is a chronic, inflammatory disease with cyclical patterns of progression and resolution at any given site. The multifactorial nature of periodontitis is based on the complex interactions between microorganisms in the microbial dental plaque, namely dental biofilm,

host response mechanisms and environmental factors[16].

Smoking is well known to be the leading environmental factor that is closely related not only with the risk but also the prognosis of periodontitis. It is the second strongest modifiable risk factor for periodontal disease after the first one which is the microbial dental plaque. It is thought to impair the immune response which compromises the periodontal tissue's ability to heal, following a period of disease activity. The traditional diagnostic methods rely mostly on clinical parameters such as probing depth (PD) and clinical attachment level (CAL) and traditional dental radiography. All clinical diagnostic techniques provide information about past disease activity and are unable to diagnose present disease activity[17].

For the objective of distinguishing between disease active and inactive sites the biochemical parameters like ALT (Alanine transaminase), Lactate dehydrogenase, ALP (Alkaline phosphatase), ß-Glucoronidase, AST, Aryl sulphatase and Neutral protease activity may be used as biomarker. Among the several host enzymes proposed as diagnostic indicators of periodontal status, ALP was one of the first to be identified. It is most effective in an alkaline environment. The optimal pH for the activity of ALP is 8.0-8.5 depending on the source[18]. ALP is stored in specific granules and released from polymorphonuclear cells (PMNs) during inflammation and from osteoblasts and periodontal ligament fibroblasts during bone formation and periodontal regeneration respectively.

Ishikawa and Cimasoni identified the potential of ALP as an important biochemical marker of gingival crevicular fluid (GCF). They quantified ALP in GCF using the colorimetric PNPP method and demonstrated that the levels of enzyme in GCF are three times greater than those in serum. They also showed a significant correlation between ALP concentration in GCF and pocket depth. This indicates that periodontal bone is also a possible source of enzyme. It is indeed known that ALP is highly active in this tissue[19].

The present study compared the clinical parameters, GCF Alkaline phosphatase levels and radiographic alveolar bone loss in smokers and non-smokers with chronic periodontitis. Patients belonging to the same age group (40-65 years) with no other known systemic problems were included in the study to nullify the effect of other contributing factors. Former smokers were excluded from the study to eliminate any long-term effect of smoking on periodontal tissues. Females were purposely excluded from the study to avoid the response bias and there is also very low prevalence of smoking present in females and hormonal changes in females can also give the false interpretation. Since patients with any known systemic problems were not included, it was considered reasonable that comparisons made between smokers and non-smokers group accurately reflected the influence of smoking on periodontium.

In this study, there was statistically highly significant difference (p value = 0.006) present in the plaque scores between the smokers and non-smokers which can be attributed to personality traits leading to decreased oral hygiene habits and/or increased rates of plaque formation in smokers. It has also been found that the toothbrushing efficiency in smokers is much less and the calcium concentration in dental plaque of smokers is found to be significantly higher than in non-smokers. The results of the present study are in accordance with various studies[20,21,22] who also showed increased levels of plaque in smokers as compared to non-smokers.

Also, there was statistically significant difference present in the gingival index scores (p value = 0.047) and the gingival bleeding index (p value = 0.049) between the smokers and non-smokers with higher values in the non-smokers when compared with smokers. The development of gingival redness was lower in smokers, suggesting a suppression of the normal inflammatory response to plaque. This may be due to tobacco smoke products such as nicotine interfering with the vascular inflammatory response. It has been found that nicotine has a potential vasoconstrictive effect. Nicotine from cigarette smoke stimulates the sympathetic ganglia to produce neurotransmitters including catecholamines. These affect the alpha receptors on blood vessels which in turn cause vasoconstriction and affect the periodontal tissues. Studies have suggested that nicotine increases rate of proliferation of gingival epithelium, thus increasing

epithelial thickness among smokers. Also tobacco use has been associated with reduced permeability of peripheral blood vessels. The results are in accordance with the studies done by Maddipati Sreedevi et al[5], Lubaba A[20], Ebru Olgun et al[22] who found less gingival inflammation and bleeding in smokers as compared to non-smokers. **Bergstrom *et al***[23] have also found less gingival bleeding in smokers than in nonsmokers, due to vasoconstriction of gingival vessels, but may also be attributable to the heavier keratinization of the gingiva in smokers.

As shown in **Table 2**, there was statistically significant difference present between the probing depth score in smokers and non-smokers (p value = 0.022). The mean levels of probing depth- scores were higher in smokers when compared with that of non-smokers.

As shown in **Table 3**, there was statistically significant difference present between the clinical attachment loss in smokers and non-smokers (p value = 0.036). The mean levels of clinical attachment level were higher in smokers when compared with that of non-smokers. The increased PD and CAL levels in smokers may be dependent on accumulation of dental plaque and poor oral hygiene. However, it was shown that deleterious effects of smoking on periodontium resulted not only from plaque amount and poor oral hygiene, but also from the effect of direct tissue destruction of smoking in homogenous groups. Smokers diagnosed with severe forms of periodontitis were shown to have more affected teeth and a higher mean loss of periodontal attachment than non-smokers with these conditions **Bergstrom *et al*** found smokers not only to have significantly increased probing depths and alveolar bone loss, but also increased tooth mobility.

As shown in **Table 4**, there was statistically highly significant difference present in the GCF ALP scores between the smokers and non-smokers with higher values in the non-smokers when compared with smokers with a p value of <0.001. ALP is enriched in the membranes of mineralizing tissue cells (e.g osteoblasts) and is also present in PMN granules, plaque bacteria, fibroblasts and osteoclasts. **Chapple *et al***[24] suggested that host-derived ALP contributed to >80% of the enzyme in GCF in levamisole inhibition and studies on suspensions of washed plaque.

Additionally, it was demonstrated that the major source of ALP within GCF was host derived and in early inflammatory disease was likely to be of PMN origin. The lower level of ALP enzyme of smokers may be due to the effect of smoking on the functions and products of PMN, which are main sources of ALP.

The results of the study are in accordance with the studies done by **Dahiya Swati *et al***[25] **and Ebru Olgan**[26] who demonstrated that GCF ALP were higher in non-smokers as compared to smokers with chronic periodontitis. Similar results were found by study done **Nishant Agrawal *et al***[27] **and Mohammad Ketabi *et al***[28].

ALP is released from polymorphonuclear cells (PMNs) during inflammation and from osteoblasts and periodontal ligament fibroblasts during bone formation and periodontal regeneration respectively. ALP activity in serum has been extensively studied, and it was suggested that ALP allows bone mineralization by releasing an organic phosphate that contributes to the deposition of calcium phosphate complexes into the osteoid matrix.

ALP might also promote mineralization by hydrolyzing inorganic pyrophosphate, a potent inhibitor of hydroxyapatite crystal formation and dissolution, within the extra-cellular calcifying matrix vesicles. Activation of osteoblasts and fibroblasts proliferation occurs through fibroblast growth factor, platelet derived growth factor and insulin like growth factor. The insulin like growth factor induces not only osteoblast growth but also ALP enzymes. At sites undergoing remission there is increased osteoblastic activity and ALP is an enzyme of osteoblast. Furthermore, among the various periodontopathogenic bacteria *Prevotella intermedia* and *Porphyromonas gingivalis* are known to have high ALP activity adding to the total ALP level, and in periodontitis one of the mechanism of collagen loss is that the fibroblast phagocytize collagen fibres. The increase in the fibroblastic activity contributes to the total ALP level

As shown in **Table 5**, there was statistically highly significant difference present in the mean % of bone

loss (detected in radiographs) between the smokers and non-smokers with higher values in the smokers when compared with non-smokers with a p-value of 0.001. The results of the study are in accordance with the study done by **Guillermo M. Rosa**[29] who demonstrated significantly lower mean alveolar bone height in the smokers (P <0.01). **Montaser N. Al-Qutub**[30] found that smokers seem to have a greater bone loss compared to non-smokers. Cigarette smoking may aggravate bone loss resulting from periodontitis.

Smoking seems to disturb the balance between proteolytic and anti-proteolytic activities in periodontal tissue. **Rosa *et al***[31] reported that nicotine increased the secretion of interleukin-6 and tumor necrosis factor alpha in osteoblasts, also nicotine increased the production of tissue-type plasminogen activator, prostaglandin E2 and matrix metalloproteinase, thereby tipping the balance between bone matrix formation and resorption toward the latter process, as reported by **Katono *et al***[32].

Receptor activator of nuclear factor-kappa ß ligand (RANKL) and osteoprotegerin (OPG) are members of the tumor necrosis factor super family. RANKL promotes osteoclastic differentiation and activates bone resorption. In contrast, OPG inhibits osteoclasto-genesis and suppresses bone resorption by inhibition of RANKL. Another potential mechanism of bone loss in smokers may be the suppression of OPG production and a change in the RANKL/OPG ratio30.

Unlike serum and saliva that are commonly used sources for assessment of biomarkers, GCF is site specific, conveniently sampled, and contains components derived from both the host and the bacteria. GCF collection and analysis are not without problems. The amount of fluid available per site is generally below a microliter in volume, and the elution of potential biochemical markers from the sample filters used is often difficult. GCF needs highly sensitive techniques for quantitative analysis. Also, the chances of contamination with saliva or blood are very high.

The present study compared the clinical parameters, GCF ALP levels and radiographic alveolar bone loss between smoker and non-smoker patients with chronic periodontitis only at baseline. Thus, it does not emphasize on the difference in the treatment outcomes on the above mentioned parameters between the two groups. From this study it can be inferred that, there is more periodontal destruction in smokers with higher mean probing depth, clinical attachment level, more radiographic alveolar bone loss and lower GCF ALP levels as compared to non-smokers. Further studies in this direction with larger sample size involving post periodontal therapy assessment in comparison to pre-treatment values prove to be of immense help in management of periodontitis in future.

CONCLUSION

ALP is a valuable biomarker because it can quantitatively evaluate the inflammatory status of gingiva and periodontal tissues. Thus, it can be used as an adjunct to clinical indices of inflammation. With the advent of novel technologies such as 'lab-on-a-chip', microfluidic devices, and rapid point-of-care oral diagnostics, the generation of a periodontal disease biomarker report appears promising for future application to diagnose periodontal diseases and to prognosticate treatment outcomes of periodontal disease. However, to further validate the association between ALP levels in GCF and disease activity, there is need for further longitudinal studies with larger sample size for extended time period.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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