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EVALUATION OF SERUM AND SALIVARY ALKALINE PHOSPHATASE LEVEL IN TOBACCO CIGARETTE SMOKERS WITH CHRONIC PERIODONTITIS

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ABSTRACT

Background and Objective: Periodontitis is a highly prevalent disease, and it affects approximately 10.5% to 12% of the world's population. Tobacco smoking adversely affects periodontal health. ALP is an essential enzyme, as it is part of the normal turnover of periodontal ligament and bone homeostasis. Saliva has many advantages as a diagnostic media. With this background this study was undertaken to evaluate Serum and Salivary Alkaline phosphatase level in tobacco cigarette smokers with chronic periodontitis. **Methods:** A total sample size of 40 subjects with age range of 25-65 years were included and were divided into 2 groups. Group I included 20 male healthy never smokers as control group and Group II included 20 male subjects with the habit of tobacco cigarette smoking using more than 5 cigarettes per day with onset of minimum six months duration and above as study group. Alkaline phosphatase level in serum and saliva was estimated. The obtained data was subjected to statistical analysis using Mann Whitney test and Spearman's correlation test. **Results:** It was found that Serum ALP was higher than Salivary ALP and there was a significant correlation between Serum ALP and Salivary ALP in Group I (Control group) and Group II (Study group). Serum ALP and Salivary ALP were both increased in Group II (Study group) compared to healthy controls. In Group II (Study group) with an increase in number of cigarettes smoked and duration of smoking there was an increase in both Serum ALP and Salivary ALP. In Group II (Study group) Serum ALP and Salivary ALP were higher in generalized periodontitis than localized periodontitis indicating the severity of chronic periodontitis. **Conclusion:** We therefore recommend salivary ALP as an early potential biomarker in tobacco cigarette smokers with chronic periodontitis for diagnosis, monitoring the disease progression, prognosis and treatment outcome.

KEYWORDS : Chronic periodontitis, Tobacco Smoker, Alkaline Phosphatase, Serum, Saliva

INTRODUCTION

Periodontium is the functional unit of tissues supporting the tooth. Periodontitis is a highly prevalent disease, and it affects approximately 10.5% to 12% of the world's population. Chronic periodontitis has been defined as an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss. Destruction of the osseous support of the dentition is a hallmark of periodontal diseases. Etiology is multifactorial origin, generally occurs due to an imbalance between pathogenic microbes, with local and systemic host response.[2] Severe and prolonged periodontal inflammation leads to loss of teeth, thereby affecting oral functions. However, in the presence of systemic or environmental factors that may modify the host response to plaque accumulation, such as diabetes, smoking, or stress, disease progression may become more aggressive.

Tobacco smoking is a risk factor for many diseases, and mounting evidence suggest that smoking adversely affects the periodontal health. Smoking creates an environment that favors colonization of pathogens. It results in increased periodontal destruction by altering the host response through impairment in neutralizing infection and this alteration results in destruction of the surrounding periodontal tissues.[2]

The term biomarker refers to biologic substances that can be measured and evaluated to serve as indicators of biological health, pathogenic processes, environmental exposure, and pharmacologic responses to a therapeutic intervention. Among several biomarkers of periodontal disease activity, ALP, being a phenotype marker of bone turnover rate has been found to be elevated in a variety of bone disorders.

ALP is an essential enzyme, as it is part of the normal turnover of periodontal ligament and bone homeostasis.[2] It is a membrane bound glycoprotein produced by a various number of cells, such as polymorphonuclear leukocytes, macrophages, fibroblasts, and osteoblasts, within the area of the periodontium and gingival sulcus. During the active stages of periodontitis, there will be destruction of alveolar bone osteoblasts and fibroblasts and their cell membrane which will be ruptured releasing their intracellular contents outside and ALP will be released from GCF into Saliva. The entry of ALP from the blood into the saliva is by passive ultrafiltration within the salivary glands and through the gingival crevice. Increase in the inflammation and bone turnover rate can cause increased activity of ALP.[5]

Radiographs are used to explore chronic periodontitis. Changes in the bone architecture takes time to manifest. The sensitivity of radiographs in detecting an early osseous lesion is poor.[5]

Interest in saliva as a diagnostic bio fluid has grown exceptionally in recent years, Saliva is a bio fluid that reflects substances existing in human serum and salivary samples have many advantages over serum. Saliva meets the demand of easy availability, non-invasive painless method of collection and less chance of transmitting infection. Changes in the bone turnover can be ascertained early with biochemical marker.

With the above background this study was undertaken to evaluate Serum and Salivary Alkaline phosphatase level in tobacco cigarette smokers with chronic periodontitis.

METHODOLOGY

Source Of Data:

In this study a total sample size of 40 subjects with age ranging from 25-65years, who reported to the Department of Oral Medicine and Radiology, M. R. Ambedkar Dental College and Hospital, Bangalore were selected. This study was approved by the Ethical Committee and Review Board of

B. R. Ambedkar Medical College and Hospital.

Method Of Collection Of Data:
The selected subjects were divided into 2 Groups, Group I included 20 male healthy non-smokers as control and Group II included 20 male subjects with the habit of tobacco cigarette smoking using more than 5 cigarettes per day with onset of minimum six months duration and above as study subjects.

In clinical examination presence of minimum 18 teeth in each subject was considered. Chronic periodontitis was assessed clinically by intraoral examination and radiograph. Depth of periodontal pocket was assessed with the help of William's periodontal probe. A minimum of 1 pocket with probing depth of 5mm or above was considered as chronic periodontitis. Bleeding on probing was included. CPITN index was done. Radiation protection protocol was followed and diagnosis were confirmed by taking OPG radiograph. Serum and saliva were collected from the selected individuals for evaluation.

Method Of Collection Of Samples
The procedure was explained to the patients. After obtaining prior informed written consent, a detailed case history with clinical examination of the patients was done and subsequently recorded. Blood and saliva were used as study samples.

Sampling of Blood:
2ml of the blood sample was drawn from the antecubital vein following aseptic procedure and transferred to an EDTA tube for estimation of ALP. Blood samples was labelled and transferred in an ice pack container to the lab where it was centrifuged at 3000rpm for 15minutes for separation of serum and serum ALP level was estimated.

Sampling of saliva:
Unstimulated saliva sample was collected by spitting method. The subjects were instructed to spit the saliva pooled in their mouth into a sterile container. 2ml of the saliva was collected. The container was labelled and transported in an ice pack container to the lab where it was centrifuged at 3000rpm for 15minutes to get the supernatant of saliva and salivary ALP level was estimated.

Biochemical Analysis:
Alkaline phosphatase level was estimated in semiautomated biochemistry analyzer based on spectrophotometric principle by kinetic method by International federation of clinical chemistry (IFCC) using alkaline phosphatase Commercial kit. The obtained values will be expressed in international units / liter.

Statistical Analysis:
The obtained data of Serum and Salivary Alkaline phosphatase were subjected to statistical analysis using Mann Whitney test and Spearman's correlation test.

DISCUSSION

Periodontal disease is defined as an inflammatory destruction of periodontal tissue and alveolar bone supporting the teeth. Although periodontitis is an infectious disease of gingival tissue origin, changes that occur in the bone are crucial as the alveolar bone destruction is responsible for tooth loss. The most common cause of alveolar bone destruction in periodontitis is the extension of inflammation from the marginal gingiva to the underlying periodontal tissues. Traditional methods for periodontal disease diagnosis such as probing depth (PD), attachment level (AL), and gingival recession, diagnose the pathology only after the disease process and the damage associated with it has already occurred.

Tobacco Smoking is an established risk factor for periodontal disease, with increased odds of disease progression in smokers. Tobacco smoking adversely affects many host systems, both local and systemic, which may account for its deleterious effects on periodontal health. Local effects may be mediated by cytotoxic and vasoactive substances in tobacco smoke, including nicotine. Systemic effects of cigarette smoking on the host immune and inflammatory responses include inhibition of peripheral blood and oral neutrophil function, reduced antibody production, and alteration of bronchoalveolar and peripheral blood immunoregulatory cell subset ratios. Nicotine metabolites which act directly as local irritants on the gingival and alveolar bone causing vasoconstriction and subsequently leading to periodontal destruction.

Nicotine interacts with the nicotinic acetyl choline receptors and stimulates the dopaminergic transmission. This in turn stimulates the reward centre and is responsible for the mood elevation and apparent improvement in cognitive function. With chronic stimulation by nicotine the GABA-ergic neurons are desensitized and thus lose their inhibitory effect on dopamine. This in turn reinforces the addiction by inducing craving.

Alkaline phosphates (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules and is a marker of bone metabolism. ALP is a highly significant enzyme in the periodontium as it is a part of the normal turnover of the periodontal ligament, root cementum, and bone homeostasis. In periodontitis, one of the mechanisms of collagen loss is that fibroblasts phagocytize collagen fibers which contributes to the total ALP level.

Gibert predicted ALP as an indicator for future loss of periodontium. It may serve as marker in periodontal treatment planning and monitoring. Its level may also be useful as a potential bone turnover marker to establish the diagnosis and prognosis of periodontal disease.

Saliva is a multifactorial biologic fluid secreted by the salivary glands and plays an important role in oral and systemic health. It is considered as the mirror of body health and is composed of variety of analytes from systemic sources that reach the oral cavity through various pathways. Various components of saliva are either passively diffused or actively transported directly from the serum into the saliva through the oral mucosa and/or gingiva, and salivary glands.

There are compelling reasons for exploring saliva as a diagnostic tool. It clearly meets the demands for a non-invasive, and easy-to-use screening method. As a diagnostic specimen, saliva has many advantages in terms of collection, storage, shipping, and voluminous sampling; all of these processes can be carried out very economically compared with serum. Saliva is also easier to handle during diagnostic procedures than blood because it does not clot, thus reducing the number of manipulations required. Moreover, a salivary test is safer than using serum, which is more likely to expose operators to blood-borne diseases. For the patients or examinees, the non-invasive painless collection approach could dramatically reduce anxiety and discomfort.

AGE
In this study distribution of patients were in the wide range of 25 – 64 years.

In Group I subjects mean age was 37.50 ± 9.71 SD. In Group II subjects mean age was 39.55 ± 11.27 SD. In the present study we found middle age subjects in Group II. Subjects for group I were age matched. These findings are in agreement with findings of Agrawal N et al, Himabindu Lalkota et al, Jeyasree RM et al.

This could be due to increased social encounters, financial independency and economic liberty they get at this age.

Gender

In Group I there were 20 (100%) Male subjects. In Group II there were 20 (100%) Male subjects. In the present study, all subjects were males. This finding is consistent with findings of Himabindu Lalkota et al.

Male predominance can be due to easy accessibility and liberty for males to use smoking.

Comparison Of Mean Serum Alp And Salivary Alp [u/l] Levels In Group I (Control group)

In Group I (Control group), the mean serum ALP (83.55 ± 33.91) was found to be higher than mean salivary ALP (21.30 ± 2.01). This correlation was statistically significant. ($p < 0.001$)

These finding are consistent with findings of Jeyasree RM et al., S Desai et al, Saleeta Mushtaq et al, P. Koppolu et al.

Comparison Of Mean Serum Alp And Salivary Alp [u/l] Levels In Group II (Study group)

In Group II (Study), the mean serum ALP (109.85 ± 28.92) was found to be higher than mean salivary ALP (31.45 ± 9.62). This correlation was statistically significant. ($p < 0.001$)

These finding are in agreement with findings of Jeyasree RM et al., S Desai et al, Saleeta Mushtaq et al,

Comparison Of Mean Serum Alp Levels Between Group I (control Group) And Group II (Study group)

In Group I, the mean serum ALP level was 83.55 ± 33.91 SD and in Group II the mean serum ALP level was 109.85 ± 28.92 SD. The mean serum ALP level was found to be higher in Group II when compared to that of Group I. The difference was found to be statistically significant ($P < 0.002$). This finding is consistent with findings of Jeyasree RM et al.

Alkaline phosphatases are membrane-bound ectoenzymes that hydrolyze monophosphate esters at a high pH (pH 8-10). Alkaline phosphatases are classified as tissue-specific and tissue-nonspecific types. ALP found in the intestine, placenta, and germinal tissue are tissue-specific. This means they are found only in the tissues where they are expressed in physiological conditions. The tissue-nonspecific alkaline phosphatases form most of the fraction circulating in serum. ALP allows bone mineralization by releasing an organic phosphate that contributes to the deposition of calcium phosphate complexes into the osteoid matrix. ALP also might promote mineralization by hydrolyzing inorganic pyrophosphate, a potent inhibitor of hydroxyapatite crystal formation and dissolution, within the extracellular calcifying matrix vesicles. ALP is stored in specific granules and secretory vesicles of neutrophils and is mainly released during their migration to the site of infection which contributes to increase in serum ALP.

Tobacco smoking exerts cumulative detrimental effects on periodontal disease progression. The host bacterial interactions normally seen in chronic periodontitis are altered, resulting in more aggressive periodontal breakdown in tobacco smokers. This imbalance between bacterial challenge and host response may be due to change in the composition of the subgingival plaque, with increase in the number and virulence of pathogenic organisms. Nicotine present in tobacco impairs the neutrophils by altering the chemotaxis, phagocytosis, oxidative burst and specially reducing the immunoglobulin G2 due to alteration in the immune inflammatory response to bacterial challenge. The vasoconstrictive properties of nicotine conceal the inflammatory and destructive changes occurring in the periodontium. Nicotine acts on the osteoclast cell by stimulating the proteinase cathepsin K, and matrix metalloproteinase 9 (MMP9) in the low pH & also activate the matrix metalloproteinase 9 on the osteoblast and subsequently lead to the bone resorption.

Comparison Of Mean Salivary Alp Levels Between Group I (control Group) And Group II (Study group)

In Group I, the mean salivary ALP level was 21.30 ± 2.01 SD and in Group II the mean salivary level was 31.45 ± 9.62 SD. The mean salivary ALP level was found to be higher in Group II when compared to that of Group I. The difference was found to be statistically significant ($p < 0.001$). This finding is consistent with findings of S Desai et al, Saleeta Mushtaq et al,

ALP is a membrane bound glycoprotein produced by many cells within the area of periodontium and gingival crevice. It is released from polymorphonuclear neutrophil during inflammation, osteoblast during bone formation and periodontal ligament fibroblast during periodontal regeneration. Thus, it has dual involvement in the process of periodontal inflammation and healing/ regeneration.[3] ALP is an important enzyme as it is part of normal turnover of periodontal ligament, root cementum and maintenance, and bone homeostasis.[5] Some forms of enzyme are also produced by plaque bacteria. ALP is stored in specific granules and secretory vesicles of neutrophils and is mainly released during their migration to the site of infection.[3]The entry of ALP from the blood into the saliva is by passive ultrafiltration within the salivary glands and through the gingival crevice.

During the active stages of periodontitis, there will be destruction of alveolar bone osteoblasts and fibroblasts and their cell membrane which will be ruptured releasing their intracellular contents outside. Therefore, ALP will be released into GCF and saliva, thereby increasing the level of ALP in saliva.

Tobacco smoking exerts cumulative detrimental effects on periodontal disease progression. The host bacterial interactions normally seen in chronic periodontitis are altered, resulting in more aggressive periodontal breakdown in tobacco smokers. This imbalance between bacterial challenge and host response may be due to change in the composition of the subgingival plaque, with increase in the number and virulence of pathogenic organisms. Nicotine present in tobacco impairs the neutrophils by altering the chemotaxis, phagocytosis, oxidative burst and specially reducing the immunoglobulin G2 due to alteration in the immune inflammatory response to bacterial challenge.

The vasoconstrictive properties of nicotine conceal the inflammatory and destructive changes occurring in the periodontium. Nicotine acts on the osteoclast cell by stimulating the proteinase cathepsin K, and matrix metalloproteinase 9 (MMP9) in the low pH & also activate the matrix metalloproteinase 9 on the osteoblast and subsequently lead to the bone resorption.

This finding is contrary to that of Kibayashi et al. found that decrease in ALP activity due to impaired neutrophil function in smokers leading to impairment of an inflammatory response in the development of periodontitis in current smokers than non-current smokers.

Correlation To Estimate The Relationship Between Serum And Salivary Alp Levels In Group I (control Group) And Group II (Study group)

In Group I and Group II the Serum ALP and Salivary ALP level shows a weak correlation coefficient range with a rho value 0.24 and 0.67 and was found to be statistically significant ($p < 0.001$).

Distribution Of Tobacco Smoking Characteristics Among Group II (Study Group)

In Group II (Study Group) out of 20 (100%) subjects, number of subjects categorized based on number of cigarettes smoked/day were 9 (45%) < 5 nos., 9 (45%) 6-10nos, 2 (10%) >10nos.

Distribution Of Tobacco Smoking Characteristics Among Group II (Study Group)

In Group II (Study Group) out of 20 (100%) subjects, number of subjects categorized based on duration of smoking were 11 (55%) for 1-5 years, 2 (10%) for 6-10 years, 5 (25%) for 11 -20 years, 2 (10%) for >20 years.

Correlation To Estimate The Relationship B/w Tobacco Smoking Characteristics And Serum & Salivary Alp Levels In Group II (study Group)

In Group II, there was moderate correlation between number of cigarettes smoked, duration of smoking in years and serum ALP & salivary ALP which was statistically significant.

Distribution Of Periodontitis Among Group II (study Group)

In Group II (Study group), out of 20 (100%) subjects, number of subjects categorized based on distribution of Periodontitis, 2(10%) had Localized Periodontitis and 18 (90%) had Generalized Peridontitis.

Mean Serum Alp [u/l] Levels Based On Distribution Of Periodontitis In Group II (study Group)

In Group II, The serum ALP levels categorized based on periodontitis cases were 2(10%) Localized periodontitis 108.00 ± 31.11 SD and 18 (90%) Generalized periodontitis 110.06 ± 29.62 SD. The mean serum ALP level was found to be higher in cases with generalized periodontitis when compared to cases with localized periodontitis. There was moderate correlation which was statistically significant (p-0.89).

These findings are consistent with findings of Himabindu Lalkota et al.

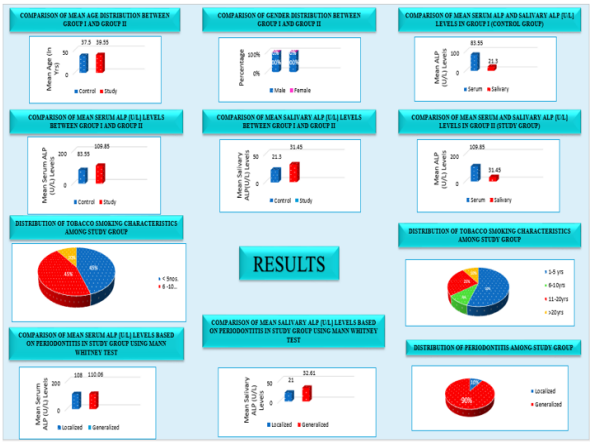
Mean Salivary Alp [u/l] Levels Based On Distribution Of Periodontis In Group II (study Group)

In Group II, The mean salivary ALP levels categorized based on periodontitis cases were 2(10%) Localized Periodontitis 21.00 ± 1.41 SD and 18 (90%) Generalized Periodontitis 32.61 ± 9.44 SD. The mean salivary ALP level was found to be higher in cases with Generalized Periodontitis when compared to cases with Localized Periodontitis. The difference was found to be statistically significant (p-0.04).

CONCLUSION

In the present study it was found that Serum ALP was higher than Salivary ALP and there was significant correlation between Serum ALP and Salivary ALP in both healthy controls and the tobacco smokers with chronic periodontitis. Serum ALP and Salivary ALP were both increased in tobacco smokers with chronic periodontitis compared to healthy controls. With an increase in number of cigarettes smoked and duration of smoking there was an increase in both Serum ALP and Salivary ALP in tobacco smokers with chronic periodontitis. Serum ALP and Salivary ALP were higher in generalized periodontitis than localized periodontitis indicating the severity of chronic periodontitis. We therefore recommend salivary ALP as a potential biomarker in tobacco cigarette smokers with chronic periodontitis.

Periodontitis is a progressive disease of the supporting structures of the teeth which can result in loss of teeth, thereby affecting important oral functions such as mastication, speech, facial esthetics and also leads to nutritional deficiency. Nicotine in tobacco smoking creates a brief feeling of contentment and pleasure that drives the desire for addiction in smoking which causes further destruction of the alveolar bone and aggravates periodontitis. Since nicotine obscures clinical signs of periodontitis and bone destruction is seen radiographically only later in the course of the disease, ALP can be an early marker of periodontitis. Saliva as a diagnostic media is an excellent choice because of its many advantages over serum. Saliva collection is simple, noninvasive, painless with no discomfort or anxiety and safe for disease detection and monitoring treatment. In the present study we found increased serum ALP and salivary ALP with significant correlation between them in tobacco smokers with chronic periodontitis. Hence Salivary ALP can be used as an early potential biomarker in tobacco cigarette smokers with chronic periodontitis in diagnosis, treatment planning, monitoring treatment outcome and prognosis. Further studies with larger sample size and inclusion of newer approaches with advanced technology is suggested.



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