**Alkaline Phosphatase and Acid phosphatase levels in saliva and serum in healthy, gingivitis and periodontitis patients before and after Scaling & Root Planing.**

**Abstract:**

Periodontitis is commonly diagnosed on the basis of clinical parameters. However, analysis of few biomarkers which are unique during the disease process and which are readily present in the saliva, blood can further help in the estimation of the rate of disease progression.

**Aim:** To correlate the association of alkaline phosphatase (ALP) and acid phosphatase (ACP) levels in saliva and serum in comparison with healthy, gingivitis and chronic periodontitis patients.

**Materials and Methods**: A total of 135 subjects with age range of 20-55 years were included in the study. The subjects were divided into 3 groups as healthy (Group A), gingivitis (Group B) and Chronic Periodontitis (Group C) with 45 subjects in each group. Clinical parameters are recorded using Silness and Loe plaque index (PI), Loe and Silness gingival index (GI) and probing depth (PD). Unstimulated saliva was collected by spitting method and blood from antecubital fossa by venipuncture. Saliva and serum were analyzed for ALP and ACP levels using auto analyzer.

**Results**: Clinical parameters showed a statistically significant decrease in the PI and GI in both Group B and Group C after SRP. There was no significant change in the PD and AL levels in the periodontitis group after SRP. Mean salivary ALP levels showed a statistically significant decrease in Group B after SRP. There was no significant decrease in the mean salivary and serum ACP levels.

**Conclusion: S**erum and salivary ALP and ACP levels were markedly decreased in group B and group C after SRP and were positively correlated with the clinical parameters.

**Keywords: Alkaline Phosphatase, Acid Phosphatase, Saliva, Serum**

**Introduction:**

Periodontitis is an infectious disease which is inflammatory in nature with complex etiology and multifactorial. The result of which is the destruction of periodontal structure and due to which there is ultimate loss of the teeth and alveolar bone. Diagnosis of periodontal disease include traditional methods such as PD, AL and gingival recession (GR). These methods diagnose the disease only after it has occurred or the damage has already taken place.

By estimating the levels of few components present in the serum or saliva can lead to prediction of the possible disease which might occur. These components might be organic, inorganic, enzymes, immunoglobulins or hormones called as biological markers or biomarkers. Biomarkers have been defined by Hulka and colleagues1 as “cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids.” The definition has been modified by Naylor2 to include biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.

Host response to periodontal disease includes production of several tissue degradation enzymes which are released from various inflammatory or bacterial cells. Among these enzymes bone markers such as alkaline phosphatase (ALP) and acid phosphatase (ACP) play an important role as their altered levels are always seen during health or disease. 6

ALP is an enzyme found in many cells of the periodontium, including osteoblasts, fibroblasts, and neutrophils (Kinane 1997).3 It is considered as an important marker associated with bone formation. ALP is released from PMN’s during inflammation, osteoblasts during bone formation and periodontal ligament fibroblasts during periodontal regeneration. Thus, it has dual involvementin the process of periodontal inflammation and healing/regeneration. ALP allows bone mineralization by releasing an organic phosphate and by hydrolyzing inorganic pyrophosphate (Deltaban 2006).4

ACP is usually present in neutrophils and is among the enzymes associated with bone metabolism mainly with osteoclast activity. Desquamated epithelial cells, macrophages, and several bacteria, including Actinobacillus, Capnocytophaga and Veillonella also produce this enzyme. Enzyme histochemistry studies have shown that chronically inflamed gingiva has elevated levels of ALP and ACP. 7

The aim of the present study is to estimate the ALP and ACP levels in saliva and serum in gingivitis and periodontitis patients before and after SRP and its correlation with the healthy individuals.

**Materials and methods:**

The present study included a total of 135 patients of both sexes with a age group of 20-55 years attending the Outpatient department of Periodontics. There were 75 males and 60 females included in the study with 24 males and 24 females in both gingivitis and periodontitis group and 24 males and 25 females in healthy group. The study population was divided into Groups A, B, C with 48 patients each in Groups B & C and 39 patients in Group A. The inclusion criteria being, systemically healthy patients with more than 20 teeth and with an age group of 20-55 years, subjects with healthy gingiva, chronic gingivitis based on clinical appearance and periodontitis with a PD of ≥ 4 mm. Exclusion criteria, patients who are under medications for any medical conditions and drugs which interfere with blood coagulation and any history of bleeding disorders, smokers, pregnant and lactating women, patients who had undergone oral prophylaxis 6 months prior to the study and patients who did not use painkillers and antibiotics since 3 months.

The following are the groups

* Systemically healthy patients with healthy gingiva – Healthy group (Group A).
* Chronic gingivitis based on indices (GI) – Gingivitis group (Group B).
* Systemically healthy patients with probing depths of ≥ 4 mm – Chronic Periodontitis group (Group C).

Prior to the start of any clinical examination, unstimulated saliva and venous blood from antecubital fossa was collected into a sterile container and were centrifuged. Serum and saliva were separated from the centrifuged samples and the estimation of ALP and ACP was done with the help of an auto analyzer. Clinical examination included plaque index (PI) (Silness and Loe), gingival index (GI) (Loe and Silness), probing pocket depth (PPD) and Clinical attachment loss (CAL) along with thorough medical and personal history. All the subjects received SRP along with oral hygiene instructions.

Patients were recalled after two weeks after SRP and blood and saliva samples were collected to estimate levels of ALP and ACP prior to clinical examination.

**Enzyme assay for saliva and serum:**

Levels of ALP and ACP were estimated with an autoanalyzer (NexGen, Span) by using Autospan clinical chemistry reagents (Span Diagnostics, Gujarat, India). Saliva and blood were centrifuged at 3000 rpm for 10 minutes and the supernatant saliva sample and serum were added with 5µl of reagent and the values were determined and expressed as units per liter (U/L). Figure 1,2,3

**Statistical analysis:**

Statistical analysis were done using SPSS version 18. Comparison of clinical indices and parameters among gingivitis and periodontitis groups was done using independent sample t test. Comparison of ALP, ACP in serum and saliva among the three groups was done using ANOVA with post hoc Games Howell test. A p-value of <0.001 was considered as highly statistically significant.

**Results:**

For each subject, the mean of the values of the clinical indices for attachment level, PPD, plaque and bleeding along with biochemical parameter like salivary, serum ALP and ACP were assessed for 90 patients (20-55 years). Summary statistics for these means across all subjects are shown in Tables 1 and 2, Graph 1.

Mean serum and salivary ALP levels were higher in both gingivitis and periodontitis group at baseline (prior to SRP). There was a statistically significant reduction in the mean serum and salivary ALP after SRP in both gingivitis and periodontitis groups, whereas serum ACP levels were statistically significant after SRP in periodontitis group. Mean serum ALP levels were higher in periodontitis group when compared to gingivitis and healthy groups and mean salivary ALP levels were higher in the periodontitis group followed by gingivitis and healthy groups. Mean serum ACP levels were higher in periodontitis group when compared to periodontitis and healthy groups. Whereas the mean salivary ACP levels were higher in the periodontitis group followed by gingivitis and the healthy group. Tables 3 & 4, Graph 2

**Discussion:**

Periodontitis is one of the major threats to oral as well as to systemic health. The process involved in the destruction of the periodontium is highly complex and various biological mediators are involved. The present study was done to evaluate the level of hydrolytic enzymes ALP and ACP as potential biochemical markers among gingivitis and periodontitis patients. The enzyme ALP plays a role in bone metabolism.

In the periodontium, ALP is of utmost importance as it is a part of normal turnover of periodontal ligament, root cementum, and bone homeostasis. ACP is a lysosomal enzyme and has high activity in bone-resorbing cells such as osteoclast and macrophages it catalyzes a variety of challenging hydrolytic enzymes that occur in multiple molecular forms with lysosomes of cells from a variety of tissues and it could also have a bacterial origin and play a role in the formation of a pathological pocket. 8

Periodontal diseases diagnosis is completed by multiple clinical parameters evaluation. However, the analysis of saliva can contribute to the diagnosis and prognosis of the disease. Numerous markers in saliva such as intracellular enzymes (creatine kinase, lactate dehydrogenase LDH, aspartate aminotransferases ASTs and alanine aminotransferases, gamma glutamyl transferase, alkaline phosphatase ALP, and acid phosphatase ACP) have been proposed as a diagnostic test for periodontal disease and appear to be useful to test the activity of periodontal disease or to measure the effectiveness of periodontal therapy. 9

AST can help to monitor the progression of periodontal disease. These enzymes are indicators of high level of cellular damage of periodontal tissue and their increased activity in gingival crevicular fluid (GCF), and saliva occurred as a consequence of their increased release from the damaged cells of soft tissues of the periodontium and reflected metabolic changes in the inflamed gingiva. 9

Present study revealed an increase in the levels of serum and salivary ALP and ACP levels with the disease progression. This can be attributed to high levels of cellular damage of periodontal tissue and increased release of both enzymes from damaged cells of the soft tissue of periodontium into GCF and consequently into saliva.

Serum and salivary ALP and ACP levels tended to decrease after SRP in both the groups showing that with decrease in the progression of disease the markers of bone destruction also decreased which was in accordance to the results obtained by Todorvic5 et al and Mohammad et al 10 There was a statistically significant difference in the mean serum and salivary ALP levels in both gingivitis and periodontitis groups before and after SRP.

Although there was a difference in the serum and salivary ACP levels in both the groups, it did not show any statistical significance. The reduction in the levels of enzymes can be based on the element that role of scaling in removal of plaque which consists chiefly of bacteria, which may be a source for ALP and ACP in GCF and consequently into saliva; consequently, their release was reduced after treatment.

Mean serum ALP levels were higher in the gingivitis group when compared to the periodontitis group and the mean salivary ALP levels were higher in the periodontitis group when compared to the gingivitis group. Correspondingly, the mean serum ACP levels were higher in the gingivitis group when related to the periodontitis group and the mean salivary ACP levels were higher in the periodontitis group when compared to the gingivitis group but there was no statistical significance observed.

Increased ALP and ACP levels were positively correlated with the increased PI and GI scores in gingivitis and periodontitis group and the PD and AL levels in the periodontitis group. After SRP, decrease in the clinical parameters coincided with the decrease in the serum and salivary ALP and ACP levels. In the recent study by Patil et al 11 indicated that smoking has several detrimental effects on periodontal tissues. A higher level of salivary biomarkers was seen in smokers with severe periodontitis.

The present study has certain limitations, such as the data collection was confined to comparatively small sample size. This sample is only a very small proportion of the entire population.

**Conclusion:**

Results of the present study suggest that the serum and salivary ALP and ACP levels were markedly decreased in both the groups after SRP and were positively correlated with the clinical parameters. The reduction in the levels of serum and salivary ALP and ACP levels was noticed with the reduction in the disease causing factors. This reduction in the serum and salivary levels of ALP and ACP can be attributed to the tissue repair process after periodontal therapy. It can be suggested that the estimation of levels of serum and salivary ALP and ACP can be considered as a valuable diagnostic marker for diagnosis of periodontal disease further studies with larger sample need to be done.