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# Evaluation of Enzymes (ALT, AST AND ALP) In Serum and Saliva of Chronic Periodontitis Patients Before and After Non Surgical Periodontal Treatment

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**Abstract**

**Background:** Periodontal diseases are not achievable through conventional methods, so there was an increased demand for reliable diagnostic aids for detection which leads to the emergence of biomarkers in the field of diagnosis. This study compares and evaluates the enzyme levels (ALT, AST & ALP) in saliva and serum of chronic periodontitis patients before and after non surgical periodontal therapy.

**Methods:** A clinical study was conducted with a total number of 33 subjects in the age group 18 – 65. This was a cohort study, in which 33 patients with probing pocket depth more than or equal to 5mm in more than 30% sites were included. Chronic periodontitis patients (sites having more than or equal to 5mm of probing pocket depth) are treated by scaling and root planning alone. The patient was reviewed in a series of three appointments. In the first visit saliva and serum collection will be done prior to the treatment. Subjects were treated by scaling and root planning alone and are provided with oral hygiene instructions. The follow up visits were scheduled at first and third month respectively from the baseline. At each

time of visits scaling & root planning was repeated if needed, saliva and serum samples were collected and enzyme levels are evaluated.

**Results & Discussion:** The results show that there is a significant reduction in salivary enzyme levels after non surgical periodontal treatment indicating the efficacy of considering saliva as a potential diagnostic aid. Whereas the variation of enzyme levels in serum is less significant except for ALP.

**Conclusion:** With the limitations of the present study it can be concluded that salivary enzyme levels ALT,ASTand ALP can be evaluated for the diagnosis of chronic periodontitis and also for the evaluation of treatment outcomes.

**Keywords:** Alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, Non Surgical Periodontal Therapy (NSPT)

**Introduction**

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”. The characteristic feature of chronic periodontitis is its slow progression rate. Gram negative organisms are mainly associated with it.

Chronic periodontitis patients are presented with clinical features like supra-gingival and sub-gingival plaque accumulation which is consistent with periodontal destruction. Gingival inflammation is usually evident with pocket formation, loss of periodontal attachment and alveolar bone loss. The diagnosis of chronic periodontitis is made on the basis of clinical examination, dental history, radiographic examination and medical history. Radiographic examination is done to evaluate the amount of bone destruction already occurred. While the above mentioned methods took longer period of time to detect measurable changes biochemical markers can detect inflammatory changes in short period of time.

The periodontal infection includes production of several enzyme families and inflammatory markers, which are released from stromal, epithelial, inflammatory or bacterial cells. The analysis of these enzymes in salivary secretion and inflammatory markers in serum of periodontitis patients can contribute to clarification of the pathogenesis and to the improvement of making a prompt diagnosis of the periodontal disease.[1-5]

Several enzymes that are evaluated for the early diagnosis of periodontal disease includes lactate dehydrogenase (LDH), aspartate and alanine aminotransferase (AST, ALT) , creatine kinase (CK), alkaline and acid phosphatase (ALP, ACP) , and gamma glutamyl transferase (GGT).

Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules and is a marker of bone metabolism. 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[2]. ALP is very important enzyme in the periodontium, as it is part of normal turnover of periodontal ligament , root cementum and maintenance, and bone homeostasis[6].

Aspartate aminotransferase (AST) also called as glutamate oxaloacetate transaminase is a member of the transaminase family of enzymes. AST is found in many body tissues including the heart, muscles, kidney, brain, and lungs.

Alanine aminotransferase (ALT) also called as glutamate pyruvate transaminase is a member of transaminase family of enzymes. ALT is found in large amounts in the liver and trace amounts are seen in heart, muscles, kidney, lungs, and brain[2]. Several studies has revealed the relation

of AST,ALT and ALP in progression of periodontal diseases. Enzyme levels in saliva can be detected same as of serum estimation and its analysis is cost -effective.

The purpose of this study is to compare the Serum and Salivary AST,ALP and ALT levels in chronic periodontitis patients before and after non surgical periodontal therapy.

**Materials & Methods**

A clinical study was conducted at the department of periodontology, PSM College of dental Science and Research, Akkikavu, Thrissur. A total number of 33 subjects in the age group 18-65 were selected . All the subjects participated in the study were informed about the nature of the study and all the participants signed an informed consent form.

**Inclusion criteria**

- Patients within the age limit of 18-65.
- Minimum of 20 teeth should be present.
- Patients who had not received any periodontal therapy for the last 6 months.
- Patients having clinical attachment loss ≥5mm in more than 30% sites.
- Patients with probing depth of ≥ 5mm in more than 30% sites.
- Patients willing to give the informed consent and willing to comply with the study were selected.

**Exclusion criteria**

- Patients with a history of smoking.
- Alcohol abuse.
- Suffering from any systemic diseases.
- Who had taken antibiotics in the past 6 months.
- Pregnant and lactating mothers .

**Study design**

This was a cohort study, in which 33 patients with probing pocket depth and clinical attachment loss more than or equal to 5mm were included. Chronic periodontitis patients are treated by scaling and root planing alone and the patient was reviewed in the first month & third month. In the first visit saliva and serum collection will be done prior to the treatment. . The 2nd and 3rd appointment were scheduled at first and third month respectively from the baseline. In that visits scaling and root planing was repeated if needed ,saliva and serum samples were collected and enzyme levels are evaluated. Out of 33 patients one of them dropped after one month review and two of them dropped after second visit.

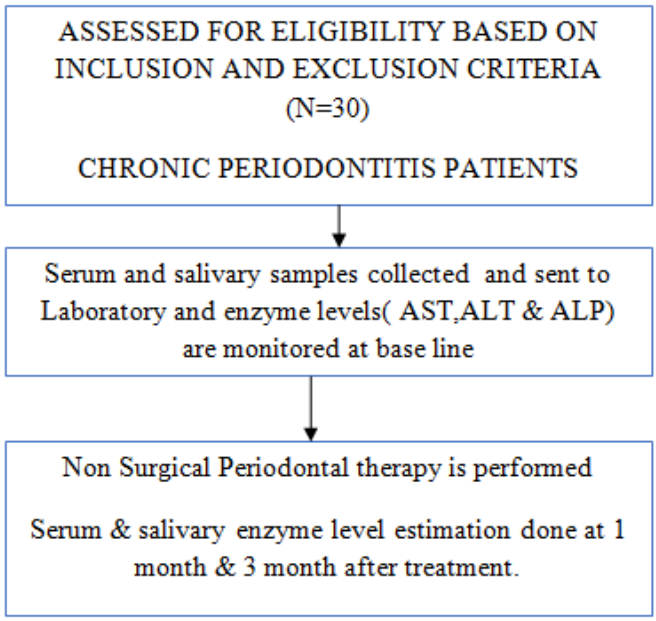


Fig 1: Flow chart showing study plan

**Collection of samples**

Subjects were asked to rinse with normal water and unstimulated saliva was collected by the spit method in a 5 ml sterile container. 5ml of random blood samples were collected from each individual, after half an hour the supernatant serum was extracted. Both samples were then placed in ice box & sent to the lab immediately then the enzyme activity in saliva was determined spectrophotometrically with the help of a semi auto analyzer.

Fig 2: Semi auto analyzer

**Non-surgical periodontal therapy**

The periodontal health was evaluated based on the probing pocket depth (PPD) and clinical attachment level (CAL).After sample collection , complete scaling and root planing is performed for all subjects. All the subjects were instructed to perform oral hygiene practices. All patients were recalled after 30 days for review and post treatment samples were collected. Also patients were again recalled after two months for review and collection of samples, scaling & root planing was repeated if it is needed.

Fig 3: Before scaling and root planing

Fig4: immediately after scaling and root planing

**Statistical analysis**

The collected data was subjected to statistical analysis through SPSS (Statistical Package for Social Science). The analysis was carried out using Wilcoxon signed Rank Test with significance level of 0.05. The Wilcoxon signed-rank test is a non-parametric statistical hypothesis test used to compare two related samples, matched samples, or repeated measurements on a single sample to assess whether their population means ranks differ.

**Results**

The mean value of enzyme levels in saliva of subjects before and after treatment is given in table 1. The results show that there is significant reduction in enzyme levels after non surgical periodontal therapy.

On comparing the mean base line value of AST levels in saliva with one month and three month post operative values, the difference in salivary AST levels between 1 month post operative values and baseline values are found to be statistically significant with p value of .006. The difference in salivary AST levels between 3 month post operative values and baseline values are found to be statistically significant with p value of .001

Table 1: Mean value of enzyme levels in saliva and serum at various periods of observations

| | Serum (Mean | Saliva (Mean) | Serum (Mean) | | Saliva (Mean) | |
|---|---|---|---|---|---|---|
| | Baseline | Baseline | One month | Three months | One month | Three months |
| AST | 32.94 | 50.18 | 30.45 | 23.87 | 39.97 | 36.3 |
| ALT | 30.45 | 39.97 | 25.58 | 25.13 | 29.29 | 22.73 |
| ALP | 23.87 | 36.3 | 186.67 | 182.43 | 62.03 | 42.97 |

On comparing the mean base line value of AST levels in serum with one month and three month post operative values , the difference in AST levels between 1 month post operative values and baseline values are found to be statistically less significant with p value of 0.458. The difference in salivary AST levels between 3 month post operative values and baseline values are found to be statistically less significant with p value of 0.054.

On comparing the mean base line value of ALT levels in saliva with one month and three month post operative values , the difference in salivary ALT levels between 1 month post operative values and baseline values are found to be statistically less significant with p value of 0.336. The difference in salivary ALT levels between 3 month post operative values and baseline values are found to be statistically significant with p value of .008

On comparing the mean base line value of ALT levels in serum with one month and three month post operative values , the difference in ALT levels between 1 month post operative values and baseline values are found to be statistically less significant with p value of 0.555. The difference in salivary ALT levels between 3 month post operative values and baseline values are found to be statistically less significant with p value of 0.35.

| | AST | | ALT | | ALP | |
|---|---|---|---|---|---|---|
| | Baseline Vs 1M | Baseline Vs 3M | Baseline Vs 1M | Baseline Vs 3M | Baseline Vs 1M | Baseline Vs 1M |
| Saliva | 0.006 | 0.001 | 0.336 | 0.0008 | 0.125 | 0.0001 |
| Serum | 0.458 | 0.054 | 0.555 | 0.35 | 0.038 | 0.003 |

Table 2: Comparison on p values of salivary enzymes at various observation intervals

On comparing the mean base line value of ALP levels in saliva with one month and three month post operative values , the difference in salivary ALP levels between 1 month post operative values and baseline values are found to be statistically less significant with p value of 0.125. The difference in salivary ALP levels between 3 month post operative values and baseline values are found to be statistically significant with p value of 0.0001

On comparing the mean base line value of ALP levels in serum with one month and three month post operative values , the difference in ALP levels between 1 month post operative values and baseline values are found to be statistically significant with p value of 0.038. The difference in serum ALP levels between 3 month post operative values and baseline values are found to be statistically significant with p value of 0.003.

**Discussion**

Many enzymes have been used as a biomarker to assess the progression of periodontal diseases. The enzyme AST is one such marker, which has been used as a diagnostic adjunct in human disease conditions such as myocardial infarction, hepatic necrosis, and many other acute, chronic, or necrotic conditions. Levels of the enzyme activity can be correlated with active tissue destruction of periodontal tissues since it is contained in the cytoplasm of cells and released on cell death. Thus, the above correlation indicates that the diagnostic test based on AST levels may be useful in assessing periodontal inflammation. ALT is a cytoplasmic enzyme and its extracellular presence is indicative of tissue cell damage. ALP activity in the periodontal ligament increases due to the constant renewal of the tissue or pathological circumstances.[2]

Previous studies have mainly investigated the activities of these enzymes in gingival crevicular fluid, which has a much closer contact with the periodontal tissues, and due to this, it surely reflects the occurrences in them much better[7]. However, the problem with the gingival crevicular fluid is that the technique of collecting it is rather complicated, and as a routine procedure, which possibly may be established, it will be hardly feasible in practice.

The activity of these enzymes can also be proved in saliva, as these enzymes are determined even in the blood of healthy persons. When a periodontal tissue becomes diseased, or its cells become damaged, due to edema or destruction of a cellular membrane, that is, of a cell as a whole, these intracellular enzymes are increasingly being released into the GCF and saliva, where their activity can be measured. Contrary to the gingival crevicular fluid there is plenty of saliva, and the procedure of its sampling is much easier and more bearable for the patient. On account of the simple and non-invasive method of collection, salivary diagnostic tests appear to hold a promise for the future.

The serum AST and ALT measured in this study did not reveal significantly different values in patients before and after non surgical periodontal therapy. This is controversial point and need evaluation in further. But the same results were obtained by Vinod K S et al in their study where they compared aspartate aminotransferases (AST) and alanine aminotransferases (ALT) levels in saliva and serum of insulin-dependent diabetes mellitus (IDDM) and normal children. They found significant difference in salivary enzyme levels but the enzyme levels in serum were found to be in significant as same as we analysed in our study [8].

The study supported the findings of Jeyasree RM et al in which they compared the quantitative levels of alkaline phosphatase (ALP) in saliva and serum before and after scaling and root planing in patients with chronic generalized periodontitis. On comparing the mean baseline salivary and serum ALP values with postoperative values in study group, the difference in salivary and serum ALP levels from baseline (79.55 ± 6.40 and 97.62 ± 4.17) to postoperative (49.47 ± 5.11 and 85.40 ± 4.10) was found to be statistically significant with $P$ = 0.000 and 0.009 for saliva and serum, respectively by them[9].

The study was in contradiction to study conducted by *Dewan and Bhatia*. In their evaluation the relationship between AST levels in saliva and gingival crevicular fluid with periodontal disease progression and their inferences showed that there was no significant difference in the level of AST in between healthy and gingivitis groups. The reason for this outcome could be related either to a low disease activity or the type of tissue affected by necrosis [10].

Various studies reported elevated levels of AST in saliva and serum of patients with periodontitis. In this study also AST levels are remarkably high for both saliva and serum at base line and the enzyme levels decreased after periodontal therapy positively for one month and three month reviews. *Sharmila J et al*. conducted a study to examine the relationship between gingival crevicular fluid (GCF) levels of aspartate aminotransferase (AST) and periodontal disease progression and to analyze the level of AST in GCF before and after the initial therapy in chronic periodontitis patients and determine the relationship between AST and conventional measures of periodontal status. There was a statistically significant difference in AST levels between diseased periodontal sites and healthy sites ($P$<0.05), and between baseline and post-initial therapy ($P$<0.05)[11].

All the studies conducted so far evaluated the presence of salivary enzymes in chronic periodontitis patients and found that there is positive correlation between enzyme levels and severity of diseases. Most of the studies were done on GCF, saliva or serum alone. Only few studies were available as a comparison between enzyme levels between saliva and serum.

**Conclusion**

From the study it is evident that Saliva has the potential to be used as the diagnostic fluid for oral disease. It's easy method of collection by non invasive way without any special equipments makes it a better choice over serum. Within the limitations of the present study it can be concluded that salivary enzyme levels ALT, AST and ALP can be evaluated for the diagnosis of chronic periodontitis and also for the evaluation of treatment outcomes. By the assessment of enzyme levels early detection of periodontal diseases is possible and disease progression can be controlled substantially.

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