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SALIVARY ENZYMES AS BIOMARKERS FOR TREATMENT OF PATIENTS WITH PERIODONTITIS: A FOLLOW-UP STUDY

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ABSTRACT: Effective treatment depends on early and successful disease detection. A timely, cost-effective diagnostic method is the principal part of the treatment plan. The invention of saliva-based microbial, immunologic, and molecular biomarkers offers novel opportunities to bypass the shortcomings of other methods by utilizing oral fluids to evaluate the condition of both healthy and diseased individuals. Periodontitis is a common oral disease characterized by inflammation, connective tissue breakdown & alveolar bone loss. One of the important features of inflammation is enzymes from different oral tissue. The aim of the study is to detect salivary & serum enzyme levels in patients with periodontitis. The aim of the present study was to evaluate salivary enzyme levels in patients with periodontitis. Our present study aims to assay the salivary Aspartate & Alanine Aminotransferase, Alkaline & Acid Phosphates levels in a patient with generalized periodontitis before & after treatment. **Material & Method:** A total of 212 participants, males & females, were selected. Group A comprised 106 healthy adults, and Group B 106 patients generalized periodontitis test group. All individuals assessed by the clinical parameters of patients within group B underwent periodontal treatment for 2 weeks. The salivary samples were collected at baseline from all groups (before done by applying student's 't' & 'z' test. **Results:** Aspartate & Alanine Aminotransferase & Alkaline & Acid Phosphates increased significantly in saliva before treatment in relation to healthy controls. After periodontal treatment, salivary enzymes ($p=0.0001$) significantly decreased. **Conclusion:** After treatment enzymes could prove patients had low aspartate & alanine aminotransferase & Alkaline & Acid Phosphates clinically indicates salivary enzymes act as biomarkers and could be used as diagnostic tool.

INTRODUCTION: The correct diagnosis and assessment of periodontal disease have acquired appreciable recognition in the last decade. The traditional diagnostic methods are probing pocket depth, clinical attachment loss, bleeding on probing, and radiographic assessment of alveolar bone loss contributing information from the past damage and hence considered as incompetent to differentiate disease activity & exactness[1]. Periodontitis is an infectious disease characterized by inflammation, connective tissue breakdown & alveolar bone loss.

A response of an organism to the periodontal infection incorporates the production of some enzyme families, which liberate from the stromal, epithelial, inflammatory, or bacterial cell [2, 19]. Host response to periodontal disease includes the production of several tissue degradation enzymes released from various inflammatory or bacterial cells, of which bone markers such as alkaline

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phosphatase (ALP) and acid phosphatase (ACP) play a crucial role as altered levels of these markers are observed in the disease. Analyzing the enzymes in salivary secretion contributes to the clarification of the pathogenesis and improvement in making a prompt diagnosis of periodontitis. These are particularly related to the group: aspartate and alanine aminotransferases (AST and ALT), alkaline phosphatase (ALP), and acid phosphatase (ACP) [2, 3].

ALP is associated with the cell plasma membrane, which transports substances from the intracellular part to the extracellular region. It is a good indicator of the metabolic activity of bone hence can be worn as a feasible biochemical marker in periodontal disease [4]. ACP a lysosomal sign, is present in neutrophils and is associated with bone metabolism. Due to the production of ACP by desquamated epithelial cells, macrophages, and several bacteria, its increased activity marks destructive processes in alveolar bone in advanced stages of the development of periodontal disease [5].

The use of saliva to measure these biomarkers (enzymes) offers several advantages. For the collection of saliva, no specialized equipment or techniques are required, it is quick and appropriate for the patient and the practitioner to collect. Additionally, whole saliva constitutes as a pooled sample with offering from all periodontal sites, and evaluation of the biomarkers in the saliva may give an overall examination of the diseases [6].

MATERIAL & METHOD: The study was conducted in the Department of Biochemistry, government medical College Miraj. Department of Biochemistry in Tatytasaheb Kore Dental College & Research Centre, New Paragon, collaborating with dept. of biochemistry DVVPFs medical college and Hospital Ahmednagar.

This study was approved by the institution's ethical committee & written informed consent was obtained from all the patients. Data about the medical & dental history were recorded. Chronic periodontitis status was determined by clinical periodontal assessments, including plaque index (PI), gingival index (GI), probing pocket depth (PPD), and Clinical attachment level (CAL). Patients included in the study were 25 to 35 years.

Group A: Healthy controls – 106.

Group B: Subjects with chronic periodontitis - 106.

Sample size Calculation: Considering the power of 80%, type – 1 error & effect size of 30, S.D. and 78.14%, the sample size was 106 in each group.

$$n = () \sigma 2 (Z\beta + Z\alpha /2)2 / (difference)2$$

$$n = (\sigma 2 (7.84) 2 / (d) 2$$

For 80%, $Z\beta = 0.84$

For a 0.05 significance level, $Z\alpha = 1.96$

r = 1 (equal number of cases & controls)

σ = S.D. of outcome Variable

d = difference of effect size)

With the above calculations, the sample size decided for this study is n = 106.

Inclusion Criteria: The following are the criteria that are included (considered) while studying the subjects:

- Clinical attachment loss ≥ 4mm. was measured by using Williams's periodontal probe.
- Periodontal Pocket depth ≥ 4mm.
- Bleeding & Probing.
- Not undergone any periodontal treatment for at least six months before sampling.
- Provide informed consent & willingness to cooperate with the study protocol.

Exclusion Criteria: The following are the criteria that are excluded while studying the subjects:

- Subjects were excluded from the study if they had a known history of cardiovascular diseases & diabetes mellitus.
- Subjects who require antibiotic or anti-inflammatory drug therapy.
- Pregnant & lactating women.

- Subjects with vitamin supplements.
- Subjects who regularly mouthwash like chlorohexidine – mouthwash.

Study Protocol: The enzymes were assessed before & after the treatment in periodontal patients and healthy control, and the processes are mentioned in this section.

Sample Collection: The saliva samples are collected first in the following way:

Saliva Collection: Samples of unstimulated mixed saliva were taken; patients were in a seated position with their heads inclined forward & the spitting method was used. Unstimulated mixed saliva was collected in a sterile test tube. After that saliva samples were centrifuged at 3000 rpm for 10-15 min. The activity of enzymes in saliva was investigated from the above samples.

Follow UP: Quantitative analysis of enzyme levels in saliva & serum in patients was estimated at baseline before treatment & after 2 weeks of periodontal treatment.

The Procedure of Estimating Enzyme Activities: The following is the procedure to estimate AST, ALT & ALP, ACP enzyme activities in saliva.

Estimation of AST Activity in Saliva:
KIT: The kit used for this process is Agappe Diagnostic Ltd.

Model: Fully Automated Biochemistry Analyzer

Method: IFCC without P-5-P Kinetic [7].

Principle: Kinetic determination of Aspartate Aminotransferase (AST) is based on the following reaction.

L- Aspartate + ά - Ketoglutarate → Oxaloacetate + L – Glutamate

Oxaloacetate + NADH + H+ → L – Malate + NDA.

AST – Aspartate aminotransferase

MDH –Malate dehydrogenase.

AST catalyses the transamination reaction at 37°C between its substrate L- aspartate & ά - ketoglutarate which yields L – glutamate & oxaloacetate. Oxaloacetate is converted into L-malate in the presence of MDH. The absorbance is measured at 340nm.

Estimation of ALT Activity in Saliva:
KIT: Agappe Diagnostic Ltd

Model: Fully Automated Biochemistry Analyzer

Method: IFCC without P-5-P Kinetic [8].

Principle: Kinetic determination of Alanine Aminotransferase (ALT) based upon the following reaction.

L- Alanine + ά - Ketoglutarate → Pyruvate + L – Glutamate

Pyruvate + NADH + H+ → L – Lactate + NDA.

ALT – Alanine aminotransferase

LDH –Lactate dehydrogenase.

ALT catalyses the transamination reaction at 37°C between its substrate L- alanine & ά - ketoglutarate which yields pyruvate & oxaloacetate. Pyruvate is converted into L- Lactate in the presence of LDH. The absorbance is measured at 340nm.

Estimation of ALP Activity in Saliva:
KIT: Agappe Diagnostic Ltd

Model: Fully Automated Biochemistry Analyzer

Method: IFCC modified method [9].

Principle: Kinetic determination of Alkaline Phosphate (ALP) based upon the following reaction.

P- nitrophenyl phosphate + H_2O ⇋ P_ Nitrophenol + Pi

Increased absorbance is directly proportional to the activity of ALP.

Estimation of ACP activity in saliva:
Method: Gutaman & Gutaman [10].

Principle: The difference between incubated and non-incubated serum liberates 1mg phenol per 100ml and M200 disodium phenyl phosphate per hour substrate pH 5 at 37°C. Colour intensities compared with standard- phenol reagent.

Statistical Analysis: Statistical software SYSTAT Version -12 (made by Crane's soft wards Bangalore) was used to analyze the data. The results were expressed in Mean ± Standard Deviation (Mean ± SD). Data were analyzed by descriptive statistics as mean, SD, percentage *etc*.

An unpaired student's t-test & was used to evaluate the significance of differences accepting P< 0.001 as the level of significance. The correlation between enzyme levels in saliva in group A & group B was done by Karl person's correction coefficient & the significance of the correlation was done by student's t-test (p<0.01) significance level.

RESULTS: Table 1, showed the clinical data & periodontal parameters of the patients & healthy controls. Mean levels of a periodontal gingival index (GI), probing depth (PD) & clinical attachment level (CAL) were significantly greater than 4mm in the group -II than in group-I

TABLE 1: CLINICAL DATA & PERIODONTAL PARAMETERS

Enzymes	Group- A (Healthy Control)	Group-B (Subjects with Periodontitis)
Gingival Index (GI)	0.61 ± 0.01	1.97 ± 0.058*
Probing depth (PD)	1 93 ± 0.09	3.63 ± 0.17*
Clinical Attachment Level (CAL) mm	2.23 ± 0.09	4.14 ± 0.19*

Table 2, illustrated that AST, ALT, ALP & ACP levels were higher in saliva in patients with periodontitis compared with healthy controls before treatment.

TABLE 2: AST, ALT, ALP & ACP IN SALIVA

Enzymes	Group- A (Healthy Control)	Group-B (Subjects with Periodontitis Before treatment)
Aspartate Aminotransferase (AST) IU/L	29.83 ± 9.30	73.30 ± 14.10*
Alanine Aminotransferase (ALT) IU/L	24.97 ± 8.80	66.20 ± 17.12*
Alkaline phosphatase (ALP) IU/L	6.39 ± 2.47	44.60 ± 11.94*
Acid phosphatase (ACP) IU/L	8.60 ± 2.47	8.60 ± 2.99*

*(p= 0.001) Considered as highly Significant

Table 3, illustrated that AST, ALT, ALP & ACP levels were decreased in saliva in patients with periodontitis compared with healthy controls after treatment.

TABLE 4: AST, ALT, ALP & ACP IN SALIVA

Enzymes	Group- A (Healthy Control)	Group-B-(Subjects with Periodontitis After treatment)
Aspartate Aminotransferase (AST) IU/L	29.83 ± 9.30	63.40 ± 14.62*
Alanine Aminotransferase (ALT) IU/L	24.97 ± 8.80	59.90 ± 14.62*
Alkaline phosphatase (ALP) IU/L	6.39 ± 2.47	31.60 ± 10.62*
Acid phosphatase (ACP) IU/L	8.60 ± 2.47	6.40 ± 2.82*

*(p= 0.001) Considered as highly Signifiant.

DISCUSSION: Unique diagnosis of periodontal tissue at risk or with a disease has been a challenge for dentists [11]. Recently some studies concentrated on investigations that have the absolute assertiveness to investigate periodontal diseases exactly. It is critical for dentists today to make reasonable decisions regarding the treatment and prevention of periodontal diseases [12].

Traditional methods such as Plaque and gingival indexes, bleeding on probing, pocket depth, and alveolar bone loss have been known for diagnosing periodontium diseases. However, they base on the disease severity than its activity. Hence, more recent methods have been contemplated to assist the diagnosis of periodontal disease activity and give information about tissues at risk of originating a new disease [13, 14].

In dentistry, these tests have become more advantageous and decisive in diagnosing, monitoring, prognosis, and screening periodontal diseases with enzymatic activity changes in the metabolic reposition in the periodontinum [4].

Saliva is an eminent diagnostic tool for the early identification of periodontitis. Numerous studies recommend that saliva is recently used as a

diagnostic tool for the early detection of periodontitis. Many tests use saliva to estimate periodontal diseases, but these tests are not utilized frequently now [4]. Samples can be readily obtained in a pain-free manner, their processing is relatively simple, their composition is less complex and they are more stable in comparison to other sources saliva also offers real-time results, being produced by exocrine glands, and therefore, yielding information on patients at the time the sample is taken [22].

Table 1 shows that the clinical data and periodontal parameters were altered. Probing pocket depth (PPD), bleeding on probing (BOP), clinical attachment loss & radiographs assessing alveolar bone level provide information on the severity of periodontitis. However, there was no measure of the disease activity.

Table 2 compares mean values of AST, ALT, ALP & ACP levels in saliva in Group A & Group B. It is seen that there was a significant increase in the mean values of AST, ALT ALP & ACP in Group A (29.83±9.30) (24.97±8.80) (6.39±2.47) (2.46±2.47). When compared with Group B (73.30 ±14.1) (66.30±9.25) (44.60±11.94) (8.60.97±2.99) before treatment. (P=0.0001).

Table 3 compares mean values of AST, ALT, ALP & ACP levels in saliva in Group A & B. It is seen that there was a significant increase in the mean values of AST, ALT ALP & ACP in Group A (29.83±9.30) (24.97±8.80) (6.39±2.47) (2.46±2.47) when compared with Group B (73.30 ±14.1) (66.30±9.25) (44.60±11.94) (8.60.97±2.99) After treatment. (P=0.0001). AST is an enzyme that can normally be found confined to the cell. After cell death takes place, it is released into the saliva during the activeness of periodontal destruction and active gingival inflammation. So, this shows a relationship between the increasing activity of AST and the presence of periodontal destruction [4, 23]. Salivary ALT enzyme is considered an intracellular enzyme and included in the cell metabolic processes, and it is mostly found in the cells of soft tissues. This enzyme is an indicator of higher levels of damage in the cell. Its increased activity in GCF is a result of its increased release from the damaged cells of the soft tissues of the periodontium. It is a reflection of the changes in the metabolism of the inflamed gingival [15]. ALP and ACP are intracellular enzymes that mainly occur in bones. Their increased concentration in saliva may be the of destructive processes in the alveolar bone in the advanced stages of evolution of periodontal disease which was proved by some former research works as well where it was determined the positive correlation between the activity of ALP and the percentage of the alveolar bone loss [16, 17, 20]. Some studies have shown a considerably increased activity of ALP in the acute phase of periodontal disease. After periodontal therapy, the activity of these enzymes renovates to the value as found in healthy persons [18, 19, 24]. This paper is a study that has shown that the increased activity of AST, ALT, ALP & ACP in saliva indicates the pathological changes located in soft tissue, primarily in the gingiva, it's an initial stage of periodontal diseases and also destructive processes [21]. That means the prognosis of the disease is much worse activity of these enzymes in saliva can be useful for the assessment of the efficiency of changing the treatment for curing periodontal diseases

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