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Research Article

SALIVARY ENZYMES AS DIAGNOSTIC MARKERS FOR DETECTION OF PERIODONTAL DISEASE

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A B S T R A C T

Introduction: The purpose of this study was to determine the salivary levels of alkaline phosphatase (ALP)
,acid phosphatase (ACP), Alanine transaminase (ALT) Aspartate transaminase , and GamaGlutamyl transferase (GGT), activities in patients with periodontal disease and to compare after the treatment and
to evaluate the use of these enzymes as biochemical markers for periodontal tissue damage.
Materials and methods: In this study, we examined the activities of salivary ALP, ACP,AST , ALT ,and GGT in patients with periodontal disease, before and after periodontal treatment. The experimental groups consisted of 20 periodontitis patients and the control group had healthy subjects (20 samples). The stimulated saliva of the patient was collected in a sterile test tube and analyzed using Erba Chem 5 semi Auto Analyzer . Periodontal disease was determined based on clinical parameters such as gingival index, probing depth and clinical attachment loss.
Results: The obtained results showed statistically significant increased activities of ALP, AST, ACP and GGT insaliva from patients with periodontal disease in relation to control group. A significant reduction in the enzyme levels was seen after conventional periodontal therapy.
Conclusion: Based on these results, salivary ALP, AST, ACP andGGT can be considered to be the biomarkers for evaluating periodontal tissue damage.

INTRODUCTION

Periodontal disease is a chronic oral polymicrobial infection characterized by periods of exacerbation and remission. It affects <50% of the adult population, and is the most common reason for adult tooth loss [1]. Periodontal disease is an important public health problem because it is preventable and treatable [2]. Periodontal disease is associated with increased risk for cardiovascular disease poorly controlled type 2 diabetes mellitus and respiratory infections, which further highlights the public health importance of good oral health [3].

The traditional diagnostic methods using clinical scores, such as, probing pocket depth, clinical attachment loss, bleeding on probing, and radiographic assessment of alveolar bone loss, give information of the past damage, and hence, have been considered inefficient to distinguish the disease activity with accuracy[4]. Recently, Analysis of the various gingival crevicular fluid biochemical markers has been proposed as a means to predict clinical attachment loss. Among the important gingival crevicular fluid components, are the various enzymes. A response of an organism to the periodontal infection includes production of several enzyme families, which are released from stromal, epithelial, inflammatory or bacterial cells. The enzymes of tissue degradation are AST (Aspartate Aminotransferase), ALT (Alanine. Aminotransferase), GGT (Gamma Glutamyl Transferase), ALP (Alkaline Phosphatase), and ACP (Acidic Phosphatase) [5].

There are a number of studies available in literature correlating the levels of these enzymes in gingival crevicular fluid (GCF) with the severity of periodontal disease [6]. However due to inherent problems in the collection of GCF, it isnot suitable for mass screening [7]. Recent studies have shown that these enzymes can be quantifiedeasily in a saliva sample [8-9]. The use of saliva to measure these biomarkers (enzymes) offers several advantages over GCF. As collection of saliva requires no specialized equipment or techniques, it is faster and more convenient for the patient and the practitioner to collect.

This present study was aimed for

1. Measurement of activities of LDH, CK, AST, ALP, ACP and GGT in saliva of healthy persons and comparison with the activities in patients with periodontal disease.

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2. Comparison of the activities of LDH, CK, AST, ALP, ACP and GGT in saliva of the patients with periodontal disease before and after treatment.

MATERIALS AND METHODS

The study was conducted in Department of Biochemistry in collaboration with Department of Periodontics, College of Dental Sci& Res Centre, Ahmedabad. Examination included 20 patients with periodontitis, and 20 healthy adult volunteers. Each subject completed a detailed medical questionnaire and received a complete periodontal examination, which included: gingival index (GI): Loe and Sillness, probing depth (PD), and clinical attachment loss (CAL).

Patients with a history of smoking, alcohol and suffering from any systemic diseases, who had taken antibiotics in the past six months or who had undergone periodontal treatment in the past six months were excluded from the study. Pregnant and lactating mothers were also excluded.

Samples of a un stimulated, mixedsaliva were taken before and after treatment, 3 minutes after mouth cleansing and before breakfast, directly from the mouth of the patient by an automatic pipette and were collected in sterile test tubes. After that, the saliva samples were centrifugedat 1000 rpm for 15 minutes. The activity of enzymes in saliva was determined spectrometrically the International Federation of Clinical Chemistry (IFCC) methodon the ERBA CHEM 5Automatic Analyzer. The determination of enzymes activity was done immediately.

Statistical Analysis

Statistical Package for the Social Sciences version 19 software was used for statistical analysis. Values of continuous variables were expressed as mean ± standard deviation (SD); p-value <0.05 was considered significant.

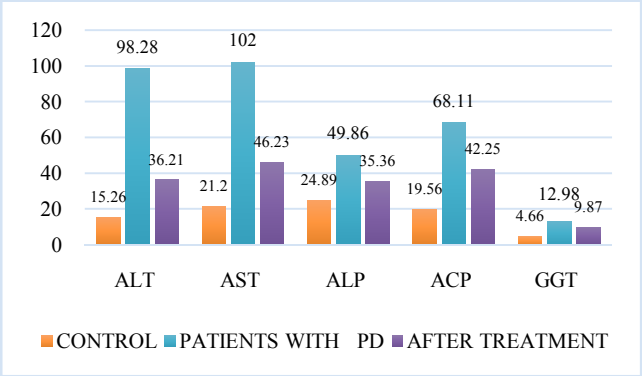
RESULTS

The obtained results showed that the activities of enzymes in saliva of the patients with periodontal disease were significantly higher in relation to the control group. The established differences showed a high level of statistical significance ($P < 0.001$) [Tables1]. After conventional periodontal treatment, the activities of salivary enzymes along with various evaluation parameters decreased significantly.

Table 1 Differences between ALT, AST, ALP, ACP and GGT activity (U/L ± SD) in saliva of healthy and patients with periodontal disease, and before and after periodontal treatment

Enzymes	Control	Patients with pd	After treatment	P value
ALT	15.26±56 U/L	98.28±6.77 U/L	36.21±13.69 U/L	<0.001*
AST	21.20±6.10 U/L	102±13.91 U/L	46.23±9.64 U/L	<0.001*
ALP	24.89±3.65 U/L	49.86±9.21 U/L	35.36 ± 7.53 U/L	<0.001*
ACP	19.56±9.13 U/L	68.11±12.88 U/L	42.25±10.04 U/L	<0.001*
GGT	4.66±0.84 U/L	12.98±2.09 U/L	9.87±4.08 U/L	<0.001*

Values are mean ± SD, as appropriate to calculate p-value, t-test used



Graph 1 Differences between ALT, AST,ALP, ACP and GGT activity (U/L ± SD) in saliva of healthyand patients with periodontal disease, and before and after periodontal treatment.

DISCUSSION

Diagnostic laboratory tests of the serum are routinely used in the evaluation of many systemic disorders. In contrast, the diagnosis of many periodontal diseases relies primarily on the clinical (GI, PD, BOP) and radiographic parameters (alveolar bone loss)[6]. But these traditional diagnostic procedures are inherently limited, in that, only the disease history and not the current disease status and the sites at risk for future periodontal breakdown, can be assessed. Advances in oral and periodontal disease diagnostic research are, therefore, moving toward methods whereby periodontal risk can be identified and quantified by objective measures such as biomarkers [10].

Numerous biomarkers in GCF have been proposed as diagnostic tests for periodontal disease. Among the intracellular enzymes that have received considerable attention as possible biomarkers of active periodontal destruction are Asparatate aminotransferase (AST), Alanine aminotransferase (ALT), Gamma glutamyl transferase (GGT), β-glucuronidase, Elastase, Alkaline phosphatase (ALP), and Acid phosphatase (ACP).

AST, ALT and GGT are intracellular enzymes included in metabolic processes of cells and they are mostly present in cells of soft tissues. These enzymes are indicators of a higher level of cellular damage and their increased activity in gingival crevicular fluid and saliva is a consequence of their increased release from the damaged cells of soft tissues of periodontium and a reflection of metabolic changes in the inflamed gingiva (11).

Previous studies mainly investigated the activities of these enzymes in the GCF, which is in a much closer contact with periodontal tissues and, due to this, it surely reflects the occurrences in them much better. However, the problem with the GCF is that the technique of collecting is rather complicated and that in a routine procedure, which possibly might be established, it would be hardly feasible in practice.

The activities of these enzymes can also be provedin saliva, as these enzymes are found even in blood of healthy persons. When a periodontal tissue becomes diseased or its cells become damaged due to edema or destruction of a cellular membrane, i.e. of a cell as a whole, these intracellular enzymes are increasingly released into the GCF and saliva where their activity can be measured. Compare to the GCF, there is plenty of saliva, the procedure of its sampling is much easier and more bearable for the patient. Being a simple and non-invasive method of collection, salivary diagnostic testsappear to hold

promise for the future. Only a few papers have focused on the activity of these enzymes in saliva in relation to gingivitis and periodontal disease and shown similar results with our study [12-13].

ALP and ACP are intracellular enzymes present in most of tissues and organs, particularly in bones.Their increased activity in saliva is probably the consequence of destructive processes in the alveolar bone in advanced stages of development of periodontal disease what 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 [14-15]. Some studies have shown a remarkably increased activity of ALP in the acute phase of periodontal disease, and after the periodontal therapy, the activity of these enzymes restored to the value as found with the healthy persons [6].This paper is a study which has shown that the increased activity of certain tissue enzymes in periodontal disease can be proved in saliva as a reflection of pathological changes in cells of periodontal tissues. The value of their activity canreflect the depth of pathological processes and damages of periodontal tissues , i.e. can show whether it is the matter of inflammation only or the destructive changes in soft tissues and bones have already commenced and can indicate the prognosis of the course of this disease.

However, the increased activity of ACP, especially ALP, indicates that the pathological destructive process has affected the alveolar bone what means that periodontal disease has significantly advanced and thus the prognosis is much worse. The activity of these enzymes in saliva can be of useful for the assessment of efficiency of changing the therapy in curing periodontal disease (16).

Previous studies mainly investigated the activities of these enzymes in gingival crevicular fluid, which is in a much closer contact with periodontal tissues and, due to this, it surely much better reflects the occurrences in them. However, the problem with the gingival crevicular fluid is in that the technique of collecting is rathercomplicated and that in a routine procedure, whichpossibly might be established, it would be hardly feasible in practice. Contrary to the gingival crevicular fluid, there is plenty of saliva, the procedure of its sampling is much easier and morebearable for the patient and, however, the same enzymes as those in the gingival crevicular fluid can be detected. Because of the simple and noninvasive method of collection, salivary diagnostic tests appear to hold promise for the future.

CONCLUSIONS

Based on the results of this study, it can be concluded that the activity levels of enzymes AST, ALT, GGT, ALP, and ACP in saliva are related to periodontal destruction. The levels of these enzymes are raised statistically in the saliva of patients with periodontitis, as compared to the controls, and its value decreases after periodontal therapy. Thus, it can be stated that the salivary enzymes can be considered as the biochemical markers of the functional condition of the periodontal tissues that provide new opportunities in making diagnoses and also evaluating the effectiveness of periodontal therapy in improving periodontal health.

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