Original article:

**Evaluating the levels of salivary enzymes as biochemical markers in periodontal disease**

*P.Krishna Kishore, **Dr. Yuvaraj Parmar, ***Satish Kumar S*

1Reader & H O D, Department of Biochemistry, College of Dental Sciences & Research Centre, Ahmadabad, India
2Sr. Lecturer Dept of Oral Medicine and Radiology, College of Dental Sciences & Research Centre, Ahmadabad.
3Sr. Lecturer, Department of Microbiology, CDS & RC
*Corresponding Author: Email: kishorepadal@yahoo.com

ABSTRACT:

Introduction: The purpose of this study was to determine the salivary levels of alkaline phosphatase (ALP), acid phosphatase (ACP), Alanine transaminase (ALT), Aspartate transaminase, Lactate dehydrogenase (LDH) and Creatine Kinase (CK), 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, LDH and CK in patients with periodontal disease, before and after periodontal treatment. The experimental groups consisted of 40 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 analysed using ErbaChem 5 semi Auto Analyser. 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, GGT and LDH in saliva 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, GGT and LDH can be considered to be the biomarkers for evaluating periodontal tissue damage.

Keywords: Acid phosphatase, alkaline phosphatase

INTRODUCTION

Periodontal disease is a chronic disease of the oral cavity comprising a group of inflammatory conditions affecting the supporting structures of the dentition. (1) The natural history of periodontitis follows a discontinuous pattern of exacerbation and remission characterized by disease activity and inactivity. (2) Periodontitis is a multifactorial disease which is affected by both genetic and environmental factors. (3) Diagnostic laboratory tests of serum are routinely used in evaluation of many systemic disorders. In contrast, diagnosis of periodontal disease relies primarily on clinical (GI, BOP, PD) and radiographic parameters (alveolar bone loss). These measures are useful in detecting evidence of past disease, or verifying periodontal health, but provide only limited information about patients and sites at risk for future periodontal breakdown. Numerous markers in saliva have been proposed as a diagnostic tests for periodontal disease such as intracellular enzymes (CK, LDH, AST, ALT, GGT, ALP, ACP). Their activity can be proved in saliva, within some normal limits, as these enzymes are determined even in blood of healthy persons. However, if a periodontal tissue becomes sick, or its cells become damaged, due to oedema or destruction of a cellular membrane, i.e. of a cell as a whole, these intracellular enzymes are
increasingly being released into the gingival crevicular fluid and saliva where their activity can be measured. Due to this, these enzymes can be biochemical markers of the functional condition of periodontal tissues (4).

So 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.
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 40 persons, of both sexes, aged 25 – 50, with periodontal disease, and 20 healthy adult volunteers. Pregnant and lactating females were excluded, post-menopausal females or others on steroid therapy were excluded. All subjects were good general health with no history of systemic disease. As the initial examination, each subject completed a detailed medical questionnaire and received a complete periodontal examination, which included: gingival index (GI), bleeding on probing (BOP), probing depth (PD). Samples of a un stimulated, mixed saliva 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 centrifuged at 1000 rpm for 15 minutes. The activity of enzymes in saliva was determined spectrometrically by the ERBA CHEM 5 Automatic Analyser. The determination of enzymes activity was done immediately.

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 ($P < 0.001$) [Tables 1 & 2]. After conventional periodontal treatment, the activities of salivary enzymes along with various evaluation parameters decreased significantly.

<table>
<thead>
<tr>
<th>ENZYMES</th>
<th>CONTROLE</th>
<th>PATIENTS WITH PD</th>
<th>AFTER TREATMENT</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>101.09 ± 12.52 U/L</td>
<td>320.23 ± 24.02 U/L</td>
<td>169.65±14.56 U/L</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CK</td>
<td>8.69 ± 1.69 U/L</td>
<td>44.25±6.25 U/L</td>
<td>23.12±3.89 U/L</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ALT</td>
<td>15.26±5.76 U/L</td>
<td>98.28±6.77 U/L</td>
<td>36.21±13.69 U/L</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>AST</td>
<td>21.20±6.10 U/L</td>
<td>102±13.91 U/L</td>
<td>46.23±9.64 U/L</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ALP</td>
<td>24.89±3.65 U/L</td>
<td>49.86±9.21 U/L</td>
<td>35.36 ± 7.53 U/L</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ACP</td>
<td>19.56±9.13 U/L</td>
<td>68.11±12.88 U/L</td>
<td>42.25±10.04 U/L</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GGT</td>
<td>4.66±0.84 U/L</td>
<td>12.98±2.09 U/L</td>
<td>9.87±4.08 U/L</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

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

CK, LDH, 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 (5).

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 proved in 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 oedema 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 tests appear 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 (6, 7).

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 (8,9 ). 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 (10). 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 can

**TABLE-2**

<table>
<thead>
<tr>
<th></th>
<th>LDH</th>
<th>CK</th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
<th>ACP</th>
<th>GGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>contrl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>after tre</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CK- creatine kinase, LDH-lactate dehydrogenase, AST-aspartate aminotransferase, ALT-alanine aminotransferase, GGT- gamma glutamyltransferase, ALP- alkaline phosphatase, ACP-acidic phosphatase.*- statistically significant difference $p < 0.001$. 

**www.ijhbr.com**  **ISSN: 2319-7072**
reflect 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. That is to say, this study shows a good correlation between the activities of CK, LDH, AST, ALT, GGT, ALP and ACP in saliva and the value of gingival index, i.e. by increasing the value of gingival index, the activity of the above mentioned enzymes was linearly increasing. This could be also stated on the basis of the typical enzyme profile in periodontal disease in relation to the healthy persons. The increased activity of CK, LDH, AST, ALT and GGT indicates the pathological changes located in soft tissues only, primarily in gingiva what could coincide with the initial stage of periodontal 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 (11). 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 rather complicated and that in a routine procedure, which possibly 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 more bearable for the patient and, however, the same enzymes as those in the gingival crevicular fluid can be detected. Because of the simple and non-invasive method of collection, salivary diagnostic tests appear to hold promise for the future.

CONCLUSIONS

On the basis of results of this study it can be concluded that the activities of CK, LDH, AST, ALT, GGT, ALP and ACP enzymes were significantly increased in the saliva of patients with periodontal disease in relation to those healthy. This is probably a consequence of pathological processes in periodontal tissues where from these intracellular enzymes are increasingly released into the secretion which surrounds them saliva. It was also established the correlation between the enzyme activity and the value of the gingival index. After periodontal treatment the activity of examined salivary enzymes was decreased, which is probably result of periodontal tissues repair. On the basis of results of this study the salivary enzymes can be considered as the biochemical markers of the functional condition of periodontal tissues what provides new opportunities in making diagnoses and following the efficiency of curing periodontal disease.

[ Acknowledgements: Authors are very thankful to entire Department of Periodontics, College of Dental Sci& Res Centre, Ahmedabad.]

REFERENCES

8. Jalil RA, Ashley FP, Wilson RF, Wagaiyu EG. Concentrations of thiocyanate, hypochiocyante, “free” and “total” lysozyme, lactoferrin and secretory IgA in resting and stimulated whole saliva of children aged 12-14 years and the relationship with plaque accumulation and gingivitis. J Periodont Res.1993;28:130–6

Date of submission: 11 January 2014, Date of provisional acceptance: 17 Feb 2013
Date of Final acceptance: 22 March 2014, Date of Publication: 09 April 2014
Source of support: Nil; Conflict of interest: Nil