Comparison of salivary alkaline phosphatase levels among diabetics and non-diabetics with chronic periodontitis

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Abstract

Aim: To evaluate the salivary alkaline phosphatase as a marker of periodontal disease activity in diabetics and non-diabetics before and after scaling and root planing therapy. Methods: Sixteen systemically healthy and 16 type–II diabetic mellitus patients with chronic periodontitis were enrolled in this study. Measurements of clinical parameters including gingival index (GI), probing depth (PD), clinical attachment loss (CAL) and collection of unstimulated whole saliva were performed at baseline and 4 weeks after scaling. Salivary alkaline phosphatase levels were analyzed by International Federation of Clinical Chemistry method for its quantification. Results: A significant difference was seen in the mean GI score at baseline between the two groups. Mean GI, PD, CAL values significantly decreased after scaling among both groups. The salivary alkaline phosphatase levels were higher among diabetics than non diabetics at baseline, which reduced after initial periodontal therapy. The result also showed significant correlation between the clinical parameters and salivary alkaline phosphatase levels after scaling and root planing therapy among diabetic patients. Conclusions: These results documented that salivary ALP level is a clinically useful marker that has a potential utility as secondary outcome measure of successful periodontal therapy.

Key words: Alkaline phosphatase; Diabetes mellitus; Chronic periodontitis; Saliva.

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Salivary ALP in diabetics with periodontitis

Introduction

Periodontal disease is one of the common inflammatory diseases with complex etiology and multifactorial in origin (1). Periodontitis is commonly diagnosed on the basis of clinical parameters, such as periodontal pocket probing depth, clinical attachment level, bleeding on probing, and bone absorption determined by radiograph (2).

Saliva is an oral fluid and has long been used as a diagnostic tool in medicine and dentistry, and interest in it as a diagnostic medium has advanced exponentially in the last ten years (3). Salivary components for periodontal diagnosis include enzymes, immunoglobulins, hormones of host origin, bacteria and bacterial products, ions and volatile compounds. Enzymes in saliva have been studied as markers of periodontal disease, and these are derived from cells in the salivary glands, polymorphonuclear leukocytes (PMNs), macrophages from gingival sulcus or pockets, and epithelial cells from oral cavity and microorganisms (4).

Intracellular enzymes are increasingly released from the damaged cells of periodontal tissues into the Gingival Crevicular Fluid (GCF) and saliva. Several enzymes evaluated for the early diagnosis of periodontal disease are aspartate and alanine aminotransferase (AST and ALT), lactate dehydrogenase (LDH), creatine kinase (CK), alkaline and acid phosphatase (ALP and ACP), and gamma glutamil transferase (GGT) (5,6).

GCF may be a potential candidate clinical sample for the screening of periodontal disease. A specific relationship between periodontal disease and parameters related to GCF has been reported. However, there are many potential sampling sites in oral cavity and differences in results among sampling sites must be considered. In addition, the sampling technique for GCF collection is not easy and a long time is needed for sample collection (2).

Alkaline phosphatase is a membrane bound glycoprotein found on most cell membranes in the body. It is produced by many cells within the periodontal environment, the principal source being polymorphonuclear leukocytes (PMNs), bacteria within the supra and subgingival plaque and through fibroblast and osteoblast activity (7). ALP is one of the potentially powerful markers of periodontal disease activity. This was first recognized by Ishikawa and Cimasoni, who demonstrated level of ALP enzyme in GCF and showed a significant correlation between ALP concentration in GCF and pocket depth (8). Most of the studies are related to levels of ALP in GCF of patients with periodontal disease (9-11), very few studies have evaluated salivary ALP levels in chronic periodontitis (1, 2, 12).

There are many risk factors for the onset and progression of periodontal disease, and it has been established by a number of studies that diabetes mellitus is a risk factor for periodontitis; or rather periodontitis is considered as the sixth complication of diabetes mellitus, indicating a two-way relationship between diabetes mellitus and periodontitis (13).

In past literature, no study could be traced that assessed the effect of type-II diabetes mellitus on the ALP levels in saliva, and its response to initial periodontal therapy that include scaling and root planing therapy and oral hygiene instructions.

Thus, the aim of the present study was to evaluate the salivary ALP levels among diabetics and non-diabetics with chronic periodontitis before and after scaling and root planing therapy, and to assess its correlation with the periodontal parameters.

Material and methods

Subjects

The study included a total of thirty-two patients of both genders between the age group of 20-65 years, visiting the outpatient...
Salivary ALP in diabetics with periodontitis

department of Periodontics, PMNM dental college and hospital, Bagalkot, Karnataka, India. The study population was divided into two groups of, sixteen patients with type – II diabetic mellitus and chronic periodontitis and sixteen systemically healthy individuals with chronic periodontitis. All patients with diabetes were diagnosed as having type II diabetes mellitus at least one year prior to the study based on American Diabetes Association standards (14) and were being treated with stable doses of oral hypoglycemic agents and/or insulin by the physician. Patients were excluded if they had any other systemic diseases, received antibiotic therapy within preceding three months, or received periodontal therapy within previous six months. Patients in the systemically healthy group were recruited from patients seeking dental treatment in the Periodontology Department, PMNM Dental College and Hospital. These patients were free of any systemic disease, were not taking any medication, had not received antibiotic therapy within preceding three months, or received periodontal therapy within previous six months. Subjects were diagnosed as having chronic periodontitis according to the criteria by American Academy of Periodontology in 1999 (15). It was ensured that all the patients had teeth with 30% periodontal bone loss and with ≥5mm deep pockets.

Ethical clearance was granted from the institutional review board and Rajiv Gandhi University of Health Sciences, Karnataka, India and written informed consent was obtained from all the participants before enrolling into the study.

Clinical assessment

All patients were evaluated clinically at their first visit by a single examiner for their periodontal conditions by assessing gingival index (16), probing depth and Clinical attachment loss (4).

All patients received non-surgical periodontal treatment comprising oral hygiene instructions, supra and sub gingival scaling along with root planing. The recall visit was scheduled after 4 weeks for re-evaluation of clinical conditions and saliva sample collection.

Gingival index (GI), Probing Depth (PD) and Clinical Attachment Loss (CAL) were assessed using a probe at six sites around each tooth, i.e., mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual locations (4).

Study design and saliva sampling

At the baseline examination, each subject completed a detailed medical questionnaire and received periodontal examination.

One ml of unstimulated whole saliva sample was collected from all the participants by spitting method. Subjects were instructed not to take food two hours prior to saliva collection. No oral examination was carried out before sample collection. After rinsing the mouth with 15ml of plain water, patients were asked to collect saliva in the floor of the mouth and then spit on to a sterile plastic container. The sample was then sent immediately to laboratory for the estimation of salivary alkaline phosphatase levels. ALP activity remains stable at room temperature for 4 hours.

Enzyme assay for saliva

The level of ALP was estimated with an auto analyzer (RA-XT Technicon*) by using International Federation of Clinical Chemistry (IFCC) method using Erba Mannheim kit (Transasia Bio-Medicals LTD. INDIA). For analysis, each saliva sample was centrifuged at 5000 rpm for 10 minutes. Reagents were added to about 10 µl of supernatant sample and determined spectrometrically by the auto analyzer and the value of ALP estimated was expressed in units per liter of saliva (U/L) (1).

Statistical analysis

Descriptive data are expressed as mean and standard deviation. Differences in clinical parameters at the baseline and after therapy
Salivary ALP in diabetics with periodontitis

were compared by Wilcoxon matched pair test among both the study groups. Differences in salivary ALP values at the baseline and after 4 weeks were assessed by paired t-test among both the study populations. Differences in clinical parameter values and salivary ALP before and after therapy were carried out by Mann-Whitney U test between diabetics and non-diabetics.

Correlation between the clinical parameter values and salivary ALP levels at baseline and after therapy was carried out by Karl- Pearson’s correlation coefficient method.

Results

Table 1 demonstrates that, both the mean GI and CAL values (2.2 and 3.1 mm) decreased by 0.9 mm after the therapy among diabetic population while PD (3.5mm) decreased by 1mm after the therapy (P<0.01). The salivary ALP enzyme levels (103.9 U/l) reduced considerably to 53.5 U/l after scaling (P<0.01).

Table 1: Clinical parameters and salivary ALP among patients with diabetes mellitus at baseline and after scaling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (Mean±SD)</th>
<th>After therapy (4 weeks)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>2.2±0.4</td>
<td>1.3±0.5</td>
<td>0.0004</td>
</tr>
<tr>
<td>PD</td>
<td>3.5±0.6</td>
<td>2.5±0.5</td>
<td>0.0004</td>
</tr>
<tr>
<td>CAL</td>
<td>3.1±0.6</td>
<td>2.2±0.4</td>
<td>0.0015</td>
</tr>
<tr>
<td>Salivary ALP</td>
<td>103.91±25.23</td>
<td>53.50±19.60</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Wilcoxon matched pair test

In non-diabetic group, the mean GI and PD values (1.9mm and 3.5mm) were significantly reduced by 0.9mm after scaling (P<0.01). The mean CAL (3.1 mm) reduced by 0.8mm after scaling and root planing therapy (Table 2). Salivary ALP level of 97.52 U/l reduced to 42.21 U/l after therapy. There was a significant difference seen in the mean baseline GI score between diabetic and non-diabetic groups. However, there was no significant difference between the study groups for periodontal parameters after the therapy, which indicates that the clinical outcome was similar in both groups (not presented in tables).

Table 2: Periodontal parameters and salivary ALP of non-diabetes at baseline and after scaling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (Mean±SD)</th>
<th>After scaling (1 month)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>1.9±0.3</td>
<td>1.0±0.2</td>
<td>0.0004</td>
</tr>
<tr>
<td>PD</td>
<td>3.5±0.5</td>
<td>2.6±0.7</td>
<td>0.0022</td>
</tr>
<tr>
<td>CAL</td>
<td>3.1±0.5</td>
<td>2.3±0.7</td>
<td>0.0022</td>
</tr>
<tr>
<td>Salivary ALP</td>
<td>97.52 ±23.74</td>
<td>42.21±10.10</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Wilcoxon matched pair test

Table 3: Comparison of salivary ALP levels between diabetics and non-diabetics at baseline and after therapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Op</td>
<td>Diabetics</td>
<td>103.91±26.05</td>
<td>0.4633</td>
</tr>
<tr>
<td></td>
<td>Non-diabetics</td>
<td>97.52±23.74</td>
<td>0.4633</td>
</tr>
<tr>
<td>Post OP</td>
<td>Diabetics</td>
<td>53.50±20.03</td>
<td>0.0409</td>
</tr>
<tr>
<td></td>
<td>Non-diabetics</td>
<td>42.21±10.10</td>
<td>0.0409</td>
</tr>
<tr>
<td>Difference</td>
<td>Diabetics</td>
<td>49.87±25.93</td>
<td>0.5730</td>
</tr>
<tr>
<td></td>
<td>Non-diabetics</td>
<td>55.30±28.01</td>
<td>0.5730</td>
</tr>
</tbody>
</table>

Mann-Whitney U test

There was a significant difference between the two groups for post-operative salivary ALP levels (Table 3). We found that there was a significant positive correlation between the clinical parameters and salivary ALP after scaling and root planing therapy in diabetic group (Table 4).

Table 4: Correlation coefficient between clinical parameters and post-operative ALP values in diabetic and non-diabetic group

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Post op ALP (Diabetics)</th>
<th>Post op ALP (Non-Diabetics)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>0.4697*</td>
<td>0.0191</td>
</tr>
<tr>
<td>PD</td>
<td>0.6496*</td>
<td>0.1899</td>
</tr>
<tr>
<td>LOA</td>
<td>0.5873*</td>
<td>0.1857</td>
</tr>
</tbody>
</table>

*p<0.05, Pearson’s correlation coefficient

Discussion
Salivary ALP in diabetics with periodontitis

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, PD and CAL) 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 (12).

Numerous markers in saliva have been proposed to be related with periodontal disease such as CK, LDH, AST, ALT, GGT, ALP, and ACP. Their activity can be proved in saliva, within some normal limits, as these enzymes are determined even in blood of healthy persons (6, 17). Saliva is abundant, and the sampling procedure is much easier, faster and more convenient for the patient and dentist. Because of the simple and noninvasive method of collection, salivary test merits attention (5, 18).

Chapple et al suggested that host-derived ALP contributed to >80% of the enzymes in GCF. It has been demonstrated that the major source of ALP within GCF was host derived and in early inflammatory disease was likely to be of PMN origin (19). In literature, although there are several studies about association between periodontal disease and ALP level of GCF, there isn’t a study about the effect of diabetes mellitus on the level of ALP in saliva.

Diabetes mellitus is a metabolic disorder arising from insulin insufficiency, which is associated with altered activity of various enzymes, such as ALP, SGOT, and SGPT etc. Besides microvascular and macrovascular complications, diabetes mellitus increases the susceptibility to infections; particularly opportunistic microorganisms that are predominant in oral microflora. There exists a relation between injured periodontal tissues and diabetes mellitus. The injured tissues secrete ALP from the PMNs that causes destruction of connective tissue and the level of ALP activity directly correlate with the intensity of inflammatory process of periodontal tissue (20).

The present study indicates that salivary ALP levels decreased in concomitance with the clinical values (GI, PD, CAL). It has been shown that the enzyme activity reflect the level of cellular damage and metabolic changes in inflamed gingival tissues. Todorovic et al (12) examined various salivary enzymes including ALP and correlated them with GI and PD; they concluded that there exists a positive correlation between ALP and clinical parameter values. In addition, the enzyme levels decreased after scaling and root planing.

Yoshie et al (6) conducted a study on individuals with chronic periodontitis and estimated their salivary ALP and LDH before and after scaling along with root planing therapy and concluded that these salivary enzymes levels were significantly reduced after the therapy. Our study showed a positive correlation between GI and salivary ALP, which is in agreement with that of the results observed by Todorovic et al (12).

Considering that Diabetes Mellitus is a risk factor for periodontitis, we compared the clinical parameter values and salivary ALP levels between diabetics and non-diabetics. The GI, PD and CAL levels were comparatively greater among diabetics at baseline than non-diabetics. However, after therapy there was no statistically significant difference between the clinical values of two groups indicating that, the clinical outcome was similar in both groups.

The present study showed a significant higher mean salivary ALP levels among the patients with type II diabetes mellitus than non-diabetic individuals. Shaheen et al (20) found that there was a significantly greater level of the salivary ALP in subjects with type II diabetes mellitus compared to non-diabetics.

Our study showed a positive correlation between clinical parameters and salivary ALP levels after scaling in diabetic
Salivary ALP in diabetics with periodontitis

group, which may indicate that the conventional periodontal therapy significantly reduced the inflammatory process in these subjects. But the clinical outcome was similar in both the groups.

Conclusions

On the basis of the results of this study, it can be concluded that the salivary alkaline phosphatase enzyme was significantly greater in diabetes mellitus subjects with chronic periodontitis compared to systemically healthy individuals; and the periodontal treatment proved beneficial in reducing the enzyme level.

Screening of periodontal disease by measuring the salivary ALP levels may be a feasible, simple, and convenient approach that does not require expert examiners. Salivary ALP reflects the inflammation and destruction of periodontal tissues, suggesting a useful marker of diagnosis of ongoing periodontal destruction as well as may have a potential utility as a secondary outcome measure of successful periodontal therapy.

Further studies on large populations are warranted to confirm the reliability of these parameters and to ascertain the effect of scaling and root planing on glycemic control.

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Salivary ALP in diabetics with periodontitis