Assessment of some salivary enzymes levels in type 2 diabetic patients with chronic periodontitis
(Clinical and biochemical study)

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ABSTRACT

Background: Diabetic patients have been reported to be more susceptible to gingivitis and periodontitis than healthy subjects. Many intracellular enzymes like (alkaline phosphatase- (ALP), aspartate aminotransferase- (AST) and alanine aminotransferase- (ALT) that are released outside cells into the gingival crevicular fluid (GCF) and saliva after destruction of periodontal tissue during periodontitis. This study was conducted to determine the periodontal health status and the levels of salivary enzymes (ALP, AST and ALT) of the study and control groups and to correlate the levels of these enzymes with clinical periodontal parameters in each study group.

Subjects, Materials and Methods: One hundred subjects were enrolled in the study, with an age range of (35-50) years, only males were included. The subjects were divided into study groups (group-I consists of 30 patients with controlled type 2 diabetes mellitus(T2DM), group-II consists of 30 patients with uncontrolled T2DM, group-III consists of 25 patients non-diabetics, all of them have chronic periodontitis(CP) and group-IV consists of 15 apparently-systemically healthy subjects and have healthy periodontium, as control group. Unstimulated saliva samples were collected for biochemical analysis of salivary enzymes (ALP, AST and ALT). The clinical periodontal parameters including: plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL) were recorded for all subjects at four sites per tooth except third molars.

Results: All clinical periodontal and biochemical parameters were highest in uncontrolled T2DM with CP patients and all enzymes levels revealed highly significant difference between all pairs of the study and control groups except AST enzyme level which demonstrated a non-significant difference between controlled T2 diabetics with CP and non-diabetics with CP. There were weak correlations between all clinical periodontal parameters and biochemical parameters except between PPD and ALT enzyme in non-diabetics with CP group and between CAL and AST enzyme in uncontrolled T2 diabetics with CP which demonstrated highly significant strong positive correlations.

Conclusion: It was concluded that T2DM and poor glycemic control have negative impact on periodontal health status. Salivary enzymes were considered as good biochemical markers of periodontal tissue destruction and useful in diagnosis, monitoring and efficient management of periodontal diseases and T2DM.

Key words: Enzymes, saliva, type 2 diabetes mellitus, periodontal diseases. (J Bagh Coll Dentistry 2015; 27(1):138-143).

INTRODUCTION

Diabetes Mellitus (DM) is an extremely important disease from a periodontal standpoint. It is a multi-systemic metabolic disorder characterized by abnormal carbohydrate, protein and lipid metabolism, the cardinal biochemical feature of this disease is elevated levels of glucose in the blood (hyperglycemia) (1).

Chronic hyperglycemia has been closely associated with an inflammatory response that has been linked to many complications (microvascular and macrovascular complications) observed in diabetes (2-4). T2DM is the more prevalent of the two major categories of overt diabetes and accounts for 90-95% of all diabetic cases and usually has an adult onset; it combines insulin resistance (IR) and insulin secretory defect (1,3).

Periodontal diseases (PDs) are one of the most widely spread diseases of mankind; they are considered an inflammatory disorder that damages tissues. The most common form of PD is called chronic periodontitis (CP) which described as a multifactorial, irreversible and cumulative condition, initiated and propagated through the complex interactions between periodontopathic bacteria and the host defense system (4-6). So, it’s considered the most important cause of tooth loss during adulthood (7). Systemic diseases are among risk factors of PD. DM and PD are thought to be associated biologically; DM is believed to promote periodontitis through an exaggerated inflammatory response to the periodontal microflora (8).

It has been shown that uncontrolled or poorly controlled DM has the greater incidence of severe PD compared with those patients who are well controlled or have no DM, which has been more prevalent in persons with T2DM (9-11). Saliva is a complex fluid that influences oral health through specific and non-specific physical and chemical properties. When disruptions in the quality or quantity of saliva occur, there will often be detrimental effects on oral and systemic health. Saliva is a mirror of general health. The diverse salivary constituents provide sources for assessment and monitoring of health and disease states (12).
Host responses to PD include the production of different enzymes that are released by stromal, epithelial or inflammatory cells. In addition to this, various enzymes associated with cell injury and cell death can be detected in GCF and saliva. Several enzymes are evaluated for early diagnosis of PD such as ALP, AST and ALT (13-15). There has been correlation between T2DM, PD and numerous markers in saliva, such as intracellular enzymes (16). These enzymes may be used to test the activity of PDs (17).

SUBJECTS, MATERIALS AND METHOD

The subjects consisted of 100 males with age range of (35-50) years. The subjects recruited for the study were patients attending the specialized center for Endocrinology and Diabetes in Baghdad, as well as, patients from the department of Periodontics, at teaching hospital, College of Dentistry, University of Baghdad. All the individuals were informed about the purposes of the investigation and consented to its protocol. The subjects were divided into:

1) **Study group (G-I):** Consists of (30) males with CP and T2DM, well controlled, the HbA1c were <7%.
2) **Study group II (G-II):** Consists of (30) males with CP and uncontrolled T2DM (moderately and poorly controlled), the HbA1c were ≥7%.
3) **Study group III (G-III):** Consists of (25) males with CP and without history of any systemic diseases.
4) **Control group IV (G-IV):** Consists of (15) males without history of any systemic diseases and with healthy periodontium, this was defined by GI scores <0.5 (18) and without periodontal pockets or clinical attachment loss. This group represents a base line data for the levels of salivary ALP, AST and ALT.

Inclusion criteria include T2DM patients (≥5 years) on oral hypoglycemic medication, body mass index level ranges between 18.5 kg/m² - 24.9 kg/m² (19), all subjects were presenting at least 20 teeth and CP in patients was defined as the presence of at least four sites with PPD ≥ 4 mm and clinical attachment loss of (1-2) mm or greater, this made according to the international classification system for PD (20).

Exclusion Criteria include T1 and T2 diabetic patients taking insulin therapy, presence of other systemic diseases other than diabetes, presence of retinopathy, neuropathy or diabetic foot, patients who have undergone periodontal treatment and course of anti-inflammatory or antimicrobial therapy 3 months prior to the study, gross oral pathology such as oral cancer and smoking or alcohol drinking.

From each subject, (5ml) of unstimulated whole saliva was collected; The collected saliva was centrifuged at 4000 rpm for 15 minutes and then the clear supernatant saliva was collected and kept frozen and stored at -20°C until biochemical analysis of salivary enzymes.

Clinical periodontal parameters examination was performed after salivary sample collection by using Michigan O periodontal probe on four surfaces (mesial, buccal, labial, distal and lingual/palatal) of all teeth except third molar. The collected data include:-

1. Assessment of Soft Deposits by the Plaque Index System (PLI) (21).
2. Assessment of Gingival Inflammation by the Gingival Index System (GI) (18).
3. Assessment of Gingival Bleeding on Probing (BOP).
4. Assessment of Probing Pocket Depth (PPD).
5. Assessment of Clinical Attachment Depth (CAL).

For ALP enzyme analysis we used kit manufactured by (BIOMERIEUX ® Sa), while for AST and ALT enzymes analysis we used kits manufactured by (RANDOX /UK). The activity of ALP was determined by measuring its absorbance at 510 nm, while the activities of AST and ALT were determined by measuring the absorbance at 505 nm both by the spectrophotometer.

Descriptive statistics in the form of mean, standard deviation, percentage and inferential statistics in the form of Games –Howell, LSD, Chi-square and Pearson correlation were used in this study. The level of significance was accepted at P< 0.05, highly significance at P< 0.01 and non-significant at P> 0.05.

RESULTS

A-Clinical periodontal parameters:

The results showed that all clinical periodontal parameters were highest in uncontrolled T2 diabetics with CP followed by non-diabetics with CP then controlled T2 diabetics with CP except for PPD which was highest in uncontrolled followed by controlled T2 diabetics both with CP then non-diabetic patients with CP, as shown in (table -1).

Highly significant differences were demonstrated by using Chi-square test for the comparisons between each two study groups regarding the percentages of BOP sites in addition, comparisons between all pairs of the study groups revealed significant and highly significant differences (p<0.01) regarding the

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other clinical periodontal parameters except for PPD and CAL between controlled T2 diabetics with CP and non-diabetics with CP which were non-significant differences (P>0.05), as shown in (table-2).

**B- Biochemical analysis:**

The obtained results have shown that the mean concentrations of salivary enzymes (ALP, AST and ALT) were highest in uncontrolled T2 diabetics with CP followed by controlled T2 diabetics with CP then non-diabetics with CP and finally control group, as shown in (table-3). Inter groups comparisons for all enzymes levels revealed highly significant differences (p <0.01) between all pairs of the study and control groups except AST enzyme level which demonstrated a non-significant difference (P>0.05) between controlled T2 diabetics with CP and non-diabetics with CP (table-4).

**C-Correlation of ALP, AST and ALT enzymes levels with clinical periodontal parameters:**

There were weak correlations between all clinical periodontal parameters and biochemical parameters except between PPD and ALT enzyme in non-diabetics with CP group and between CAL and AST enzyme in uncontrolled T2 diabetics with CP which demonstrated highly significant positive strong correlations. Significant positive correlations were revealed between PLI and AST enzyme in controlled T2 diabetics with CP, between GI and ALP enzyme in uncontrolled T2 diabetics with CP, as well as, CAL with both AST enzyme in controlled T2 diabetics with CP and ALT enzyme in uncontrolled T2 diabetics with CP and non-diabetics with CP, as shown in (table-5).

Table 1: Statistical description (Mean ± SD) of (PLI, GI, PPD and CAL) and percentages of sites according to BOP scores for the study and control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>PLI</th>
<th>GI</th>
<th>BOP</th>
<th>PPD</th>
<th>CAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Score 0</td>
<td>Score 1</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>G- I</td>
<td>1.62 ± 0.59</td>
<td>1.61 ± 0.63</td>
<td>61.39 %</td>
<td>38.61 %</td>
<td>4.47±0.63</td>
</tr>
<tr>
<td>G- II</td>
<td>2.69 ± 0.37</td>
<td>2.79 ± 0.39</td>
<td>2.16 %</td>
<td>97.84 %</td>
<td>5.03±0.43</td>
</tr>
<tr>
<td>G- III</td>
<td>2.05 ±0.68</td>
<td>2.00 ± 0.55</td>
<td>49.76 %</td>
<td>50.24 %</td>
<td>4.42±0.56</td>
</tr>
<tr>
<td>G- IV</td>
<td>0.12 ± 0.09</td>
<td>0.09 ± 0.08</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Inter groups Comparisons of the mean values of PLI, GI, PPD, CAL and the Percentages of BOP sites between all pairs of the study groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>PLI</th>
<th>GI</th>
<th>BOP</th>
<th>PPD</th>
<th>CAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Score 0</td>
<td>Score 1</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>G- I</td>
<td>0.000</td>
<td>HS</td>
<td>0.000</td>
<td>HS</td>
<td>0.000</td>
</tr>
<tr>
<td>G- II</td>
<td>0.042</td>
<td>S</td>
<td>0.046</td>
<td>S</td>
<td>0.000</td>
</tr>
<tr>
<td>G- III</td>
<td>0.000</td>
<td>HS</td>
<td>0.001</td>
<td>HS</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 3: Statistical description (mean level in IU/L ± SD) of ALP, AST and ALT enzyme for the study and control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP</th>
<th>AST</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
</tr>
<tr>
<td>G- I</td>
<td>9.487 ± 1.983</td>
<td>14.533 ± 1.624</td>
<td>10.27 ± 0.89</td>
</tr>
<tr>
<td>G- II</td>
<td>14.587 ± 2.541</td>
<td>18.433 ± 1.165</td>
<td>13.25 ± 1.96</td>
</tr>
<tr>
<td>G- III</td>
<td>7.888 ± 1.15</td>
<td>13.56 ± 2.053</td>
<td>8.34 ± 1.24</td>
</tr>
<tr>
<td>G- IV</td>
<td>5.993 ± 0.823</td>
<td>9.533 ± 1.506</td>
<td>5.07 ± 1.52</td>
</tr>
</tbody>
</table>

Table 4: Inter groups comparisons of the mean concentrations (IU/L) of ALP, AST and ALT enzymes between all pairs of the study and control groups by using Games-Howell Method

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP</th>
<th>AST</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
</tr>
<tr>
<td>G- I</td>
<td>0.000</td>
<td>HS</td>
<td>0.000</td>
</tr>
<tr>
<td>G- II</td>
<td>0.003</td>
<td>HS</td>
<td>0.233</td>
</tr>
<tr>
<td>G- III</td>
<td>0.000</td>
<td>HS</td>
<td>0.000</td>
</tr>
<tr>
<td>G- IV</td>
<td>0.000</td>
<td>HS</td>
<td>0.000</td>
</tr>
<tr>
<td>G- III</td>
<td>0.000</td>
<td>HS</td>
<td>0.000</td>
</tr>
</tbody>
</table>
Table 5: Person's Correlation Coefficient (r) between clinical periodontal parameters and the levels of salivary enzymes for each study group

<table>
<thead>
<tr>
<th>ALP enzyme</th>
<th>PLI</th>
<th>GI</th>
<th>BOP</th>
<th>PPD</th>
<th>CAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-I</td>
<td>0.058</td>
<td>0.380</td>
<td>0.221</td>
<td>0.137</td>
<td>0.469</td>
</tr>
<tr>
<td>G-II</td>
<td>0.215</td>
<td>0.127</td>
<td>0.379</td>
<td>0.024</td>
<td>0.901</td>
</tr>
<tr>
<td>G-III</td>
<td>0.034</td>
<td>0.435</td>
<td>0.012</td>
<td>0.478</td>
<td>0.001</td>
</tr>
<tr>
<td>AST enzyme</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-I</td>
<td>0.420</td>
<td>0.010</td>
<td>-0.222</td>
<td>0.192</td>
<td>-0.099</td>
</tr>
<tr>
<td>G-II</td>
<td>0.110</td>
<td>0.281</td>
<td>0.006</td>
<td>0.487</td>
<td>-0.254</td>
</tr>
<tr>
<td>G-III</td>
<td>0.105</td>
<td>0.308</td>
<td>0.185</td>
<td>0.189</td>
<td>0.222</td>
</tr>
<tr>
<td>ALT enzyme</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-I</td>
<td>-0.125</td>
<td>0.256</td>
<td>0.047</td>
<td>0.402</td>
<td>0.114</td>
</tr>
<tr>
<td>G-II</td>
<td>-0.137</td>
<td>0.236</td>
<td>0.165</td>
<td>0.192</td>
<td>-0.050</td>
</tr>
<tr>
<td>G-III</td>
<td>0.243</td>
<td>0.121</td>
<td>-0.269</td>
<td>0.097</td>
<td>0.365</td>
</tr>
</tbody>
</table>

DISCUSSION

In the present study significant statistical differences were found between controlled T2 diabetic patients with CP and non-diabetics with CP and highly significant differences were found between uncontrolled T2 diabetics and both controlled T2 diabetics with CP and non-diabetics with CP regarding PLI and GI. These were in agreement with other studies (22-24) and in disagreement with Ibrahim and Abaas (25) and Sharma et al (26). These are explained by the fact that patients with diabetes tend to be systemically compromised and that their oral environment is also compromised due to the reduction in the buffering capacity and volume of their saliva, increased salivary viscosity and the change in bacterial flora (27, 28). All these factors lead to higher accumulation of plaque and calculus. Diabetes is often associated with increased gingival inflammation in response to bacterial plaque as the inflammatory reactions are intensified during poor metabolic control (8).

The result of this study revealed that the uncontrolled diabetics have more sites with bleeding on probing than controlled and non-diabetic groups and this was in agreement with Offenbacher et al (29) and in disagreement with Kumar et al (30). Regarding PPD and CAL the results represent highly significant differences were found between uncontrolled T2 diabetic patients with CP and both controlled T2DM and non-diabetic patients with CP. These results were in agreement with studies (31, 32) and in disagreement with other study (33). This result may be explained by the fact that poor glycemic control, with the associated increase in advanced glycation end products, renders the periodontal tissues more susceptible to destruction. The cumulative effects of altered cellular response to local factors, impaired tissue integrity and altered collagen metabolism as the collagen in diabetic patients is aged and more susceptible to breakdown (34), these undoubtedly play a significant role in the susceptibility of diabetic patients to infections and destructive PD. There were increased BOP, PPD, increased tooth mobility and greater loss of attachment as the individuals with diabetes are twice as likely to exhibit attachment loss as non-diabetic individuals (35).

Numerous markers in saliva have been proposed as a diagnostic test for periodontal disease such as (ALP, AST and ALT) enzymes. These intracellular enzymes are increasingly being released by sick periodontal tissues into the GCF and saliva where their activity can be estimated. From the present study findings revealed that the level of ALP was higher in diabetic groups than in non-diabetics and control groups. The observed high enzyme activity can be attributed to increase in inflammation and bone turnover rate as ALP enzyme is produced by polymorphonuclear leukocytes, osteoblasts, macrophages, fibroblasts and plaque bacteria within periodontal tissues or pockets. The increased ALP activity is probably a consequence of destructive process in the alveolar bone in the advanced stages of PD (13). The present findings revealed that there were highly significant differences in salivary ALP levels between all pairs of the study groups and with control group. This is in accordance with many other studies (16, 35).

The result of this study showed highly significant difference in salivary AST level between all pairs of the study groups and with control group. This is in accordance with many other studies (16, 36). Regarding ALT enzyme the results revealed that there were highly significant
differences in enzyme level between all pairs of the study groups and with control group, this was in agreement with previous studies (18,37). There were weak non-significant positive correlations between ALP and clinical periodontal parameters (PLI, BOP, PPD and CAL) in all study groups, while there is a significant positive correlation between ALP and GI in uncontrolled T2 diabetics with CP group. These results were in agreement with Kalburti et al (35) and Sanikop et al (36) and in disagreement with Kumar Sharma (39). The possible explanation of weak non-significant correlation may be due to limited human sample size. Regarding the AST enzyme there were a highly significant strong positive correlation between salivary AST and CAL within uncontrolled T2 diabetic patients with CP, and significant positive correlation between AST enzyme and both PLI and CAL parameters within uncontrolled T2 diabetic patients with CP. These findings agreed with Abdul- Hadi (40) and in disagreement with another study (15). The findings revealed that there were weak non-significant correlations between the ALT enzyme and the clinical periodontal parameters except between ALT enzyme and PPD in non-diabetics with CP which was highly significant strong positive correlation and between ALT enzyme and CAL in (uncontrolled T2 diabetics and non-diabetics) with CP which was significant positive correlations. These results agreed with Abdul- Hadi (40) and Raiet et al (41) and in disagreement with Todorovic et al (15). The significant positive correlations between ALT enzyme with PPD and CAL parameters indicated that the enzyme activity increased with increasing severity of periodontitis. An increase in AST and ALT enzymes activities reflect the negative effect of the (T2DM) on the periodontal tissue especially the soft tissue since these enzymes are intracellular included in the metabolic processes of cells and they were indicators of a higher level of cellular damage and a reflection of metabolic change in the inflamed gingiva (13,14,17).

REFERENCES

Oral and Maxillofacial Surgery and Periodontics


