Evaluation of salivary enzymes activities among patients with chronic periodontitis

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ABSTRACT
Background: The cells of periodontium contain many intracellular enzymes like (alkaline phosphatase ALP, aspartate aminotransferase AST and alanine aminotransferase ALT) that are released outside into the saliva and gingival crevicular fluid GCF after destruction of periodontal tissue during periodontitis. The aim of this study is to determine the activities of these enzymes in saliva and its relation to the clinical periodontal parameters during chronic periodontitis.

Materials and methods: Measurements of plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PDP) and clinical attachment level (CAL) were taken from sixty subjects (thirty with chronic periodontitis and thirty with healthy periodontium), only male were included and saliva was collected from them and subjected to biochemical analysis of the enzymes alkaline phosphatase ALP, aspartate aminotransferase AST and alanine aminotransferase ALT levels.

Results: Statistical analysis of the results revealed the presence of a highly significant difference in the enzymatic activity between healthy and chronic periodontitis subjects with absence of any correlation between the activities of these enzymes and the clinical periodontal parameters except between alanine aminotransferase ALT and PLI (P value :0.049) and between alkaline phosphatase ALP and BOP (P value: 0.041).

Conclusions: It can be concluded that these enzymes are good biochemical markers and helpful in early diagnosis of chronic periodontitis.


INTRODUCTION

Periodontal disease is one of the common inflammatory diseases within complex etiology and multifactorial in origin. Diagnosis of periodontal disease has been primarily based upon clinical and radiographic measures of periodontal tissue destruction. These parameters provide measures of past destruction and are of limited use in early diagnosis (1). However, advances in molecular biology and genetics are leading to a better knowledge of the pathways and mechanisms through which bacteria maintain the host immune/inflammatory response (2). New auxiliary diagnostic tools based on body fluids, such as saliva and gingival crevicular fluid (GCF), as well as studies of subgingival microflora and genetic susceptibility, are useful and should be further developed (3).

Saliva has been discussed lately as an important biological material that introduces new diagnostic tests which may contribute in the diagnosis and explaining the pathogenesis of many diseases (4).

It has been extensively studied in relation to periodontal disease because it is easily collected and allows analysis of several local and/or systemic biological markers such as proteins, enzymes, host cells, hormones, bacterial products, volatile components and ions (5). Saliva also contains many enzymes and some inflammatory markers. These enzymes in serum have been routinely examined for screening of systemic disease. Therefore, no specific laboratory devices are necessary, and this approach may be suitable for public health use (6-8).

Enzymes are biological catalysts that carry out tightly controlled biological reactions with high specificity. Like a chemical catalyst, an enzyme acts by lowering the activation energy of a reaction, thereby inducing the formation of the products from the substrates (9). Intracellular enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), acidic and alkaline phosphatase (ACP and ALP) etc. are increasingly released from the damaged cells of periodontal tissues into the saliva (5).

Such AST, ALT and ALP can help to monitor the progression of the periodontal disease. These enzymes appear to be useful to test the activity of periodontal disease (10). Due to these detectable issues, therefore we decide to study the clinical periodontal parameters and the biochemical analysis of ALP, AST and ALT enzymes in saliva.

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samples of patients with chronic periodontitis and normal healthy gingiva persons.

**MATERIALS AND METHODS**

**Human sample**

Sample population consisted of sixty individuals; only males with an age ranged between 25 to 45 years old. All individuals had no history of systemic disease. The exclusion criteria were including a course of anti-inflammatory or antimicrobial therapy within the previous three months, a history of regular use of mouth washes, use of any vitamin supplementation, smoking, mucosal lesions, chemotherapy, radiation therapy and Medications that cause xerostomia.

The sample was divided into two groups. The study group consisted of thirty males who attended the Department of Periodontology of the College of Dentistry at the University of Baghdad. All subjects had chronic periodontitis based on clinical examination. Chronic periodontitis in patients was defined as the presence of teeth with probing pocket depth >4mm with clinical attachment loss, this was made according to the international classification system for periodontal disease in 1999 (11). The control group consisted of 30 male. All of them had healthy periodontium depending on the absence of clinical signs of inflammation and using the traditional clinical periodontal parameters.

**Saliva samples collection**

The study and control subjects were instructed not to eat or drink for at least 1 hour before collection of the sample. After that, the subject rinsed his mouth several times with distilled water and then waiting for 3 minutes for water clearance. Then, each one of the study and control groups’ subjects was asked to spit his saliva into a polyethylene tube until 3-4ml was collected. After that, the sample was put in a cooling box to stop the growth of bacteria before storing at -20 °C in the freezer.

**Clinical examination**

Collected data included assessment of plaque index PLI (Silness and Loe in 1964), gingival index GI (Loe and Silness 1963), bleeding on probing BOP (Ainamo and Bay, 1975), probing pocket depth PPD (Saiko et al, 2005) and clinical attachment level CAL (Lindhe et al, 1998).

**Biochemical analysis**

For enzymes analysis we use kits manufactured by BIOLABO SA which were used routinely to measure the activities of ALP, AST and ALT in serum. The activity of ALP was determined by measuring its absorbance at 510 nm by the spectrophotometer. The absorbance at 505nm by the spectrophotometer.

**Statistical analysis**

Descriptive statistics in the form of mean, standard deviation and standard error and inferential statistics in the form of Student t-test, p-value, Pearson and Spearman correlation were used in this study.

**RESULTS**

The results of this study showed that the activities of the enzymes ALP, AST and ALT in saliva samples of patients with chronic periodontitis were higher in relation to the control group (table 1).

Statistical analysis using the student t-test revealed the presence of a highly significant difference in the activities of these enzymes between the study and the control groups (p-value< 0.001).

**Table 1: The means of ALP, AST and ALT activities in IU/L in the study and the control groups**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>33.02±5.17</td>
<td>4.62±0.66</td>
</tr>
<tr>
<td>AST</td>
<td>30.46±5.37</td>
<td>20.10±4.13</td>
</tr>
<tr>
<td>ALT</td>
<td>23.27±4.91</td>
<td>15.40±4.18</td>
</tr>
</tbody>
</table>

Regarding the correlation between the activities of these enzymes with the clinical periodontal parameters (PLI, GI, BOP, PPD and CAL), this study revealed the absence of any correlation except between alanine aminotransferase ALT and PLI (Pvalue: 0.049) and between alkaline phosphatase ALP and BOP (P value: 0.041).

**DISCUSSION**

A response of an organism to the periodontal infection includes production of several intracellular enzyme families which are released from stromal, epithelial, inflammatory or bacterial cells such as ALP, AST and ALT (12). Alkaline Phosphatases are a group of enzymes found primarily in the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2) (13). Salivary and serum ALP mean concentration was shown to be positively correlated with periodontal disease activity and is higher in individuals with periodontal diseases than periodontally healthy subjects (14). Our findings revealed that the level of Alkaline Phosphatase ALP in the study group was significantly higher than the control group. The explanation for this difference in the enzyme activity between the two groups may be due to the
fact that ALP is present at or near the cell membrane of alveolar bone osteoblasts and fibroblasts of the PDLs (15-17). During the active stages of periodontitis, there will be destruction of alveolar bone osteoblasts and fibroblasts and their cell membrane will be ruptured releasing their intracellular contents outside. So ALP will be released into saliva and GCF and the level of ALP will increase in saliva (5, 10, 18).

AST is present in the liver and cardiac cells and is most useful as a marker of liver or cardiac injury (19), while ALT is especially concentrated in the liver; it leaks out of the liver cell and increases in the serum with liver damage, as in hepatitis and mononucleosis (20). According to Kaufman and Lamster in 2000 both of these enzymes are increasingly released from the damaged cells of inflamed periodontal tissue. This study revealed the presence of a highly significant difference in the enzymatic activity of AST and ALT between the two groups. A possible explanation of this difference in the enzyme activity of AST and ALT between the two groups may be due to the fact that significant AST and ALT levels have also been found in human gingival epithelial cells, human gingival fibroblasts and human periodontal ligament fibroblasts i.e. the cells of the soft tissue (21). During periodontitis or gingivitis, the cells of the soft tissue of periodontium become damaged due to edema or destruction of the cell membrane releasing their enzymatic contents into saliva and gingival crevicular fluid GCF. So the activity of AST and ALT will increase in saliva (22, 23).

Concerning the correlation between the activities of these enzymes with the clinical periodontal parameters, our study did not show any correlation except between ALT with PLI and between ALP with BOP, while other previous studies show different correlations.

REFERENCES