Salivary Levels of Rheumatoid Factor and Anti-cyclic Citrullinated Antibody in Periodontitis

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ABSTRACT

Background: There has been growing evidence suggesting an association between periodontitis (PD) and the increased risk of systemic disease such as rheumatoid arthritis (RA). PD and RA share many pathological aspects and immunological findings.

Aims of study: This study was designed to assess the association between PD and RA by measuring the Rheumatoid factor (RF) and anti-cyclic citrullinated antibody (ACPA) in saliva of patients with chronic periodontitis.

Patients and Methods: Thirty patients with chronic PD their ages range from 25-55 years with a mean age of 39.1±4.2 years and twenty healthy individuals were studied. Salivary level of RF-IgM was estimated by agglutination test, while ACPA-IgG level was assessed by means of enzyme-linked immune-sorbent assay (ELISA).

Results: The current results showed that there is no significant differences (p>0.05) in the presence of RF between patients and controls, RF-IgM was detected in saliva of 3 patients with PD (10%) and in one case (5%) of 20 healthy control, whereas the salivary levels of ACPA-IgG in chronic periodontitis were significantly elevated as compared to healthy controls (16.29 ± 4.3 RU/ml vs.1.19± 0.9 RU/ml) respectively, (p<0.05).

Conclusion: These findings indicate that the detection of ACPA in the saliva of some PD patients may be elicited by certain micro-organisms in the subgingival plaque.

Key words: Periodontitis, Rheumatoid arthritis, ACPA and RF.

INTRODUCTION

Periodontitis is a chronic inflammatory disease where resident cells and preformed mediators induce leukocyte infiltration and progressive destruction of the tooth supporting tissues as a result of interaction between bacterial products, cell populations, and mediators in disease-susceptible individuals1. PD is an infection initiated by bacteria present in the dental biofilm, include: Porphyromonas gingivalis (P.
RA is a systemic autoimmune disease characterized by progressive joint destruction and a variety of systemic manifestations resulting from chronic inflammation. It affects approximately 1% of the adult population. RA is characterized by the inflammation of the synovial membrane, leading to an invasion of the synovial tissue into the adjacent cartilage matrix with degradation of the articular cartilage and bone. Citrullination or deamination is the term used for a genetic modification of the amino acid arginine in a protein into the amino acid citrulline and caused by enzymatic activity through Peptidyl-Arginine Deaminases (PAD) enzyme. It has been found that *P. gingivalis* is currently the only known bacterium with the expression of PAD which is involved in citrullination. ACCP are highly specific for RA and have been implicated in disease etiology, it may be detected in roughly 50-60% of patients with early RA. Studies report there is a correlation between both PD and RA since the mechanisms for the development of RA have consonance with the pathogenesis of chronic PD. Both PD and RA present an imbalance between pro-inflammatory and anti-inflammatory cytokines, which is deemed responsible for the tissue damage. In this sense, both conditions are associated with destruction of bone, mediated by inflammatory cytokines such as interleukin-1, tumor necrosis factor and prostaglandin E2. This study was designed to assess the association between PD and RA by measuring the RF and ACPA in saliva of patients with chronic periodontitis.

**SUBJECTS AND METHODS**

A total of 30 patients with chronic PD were studied, their ages range from 25-55 years with a mean age of 39.1±4.2 years. They were selected among people referring to periodontics departments in College of Dentistry, Baghdad University for diagnosis and treatment of PD from November 2012 till January 2013, who were volunteers to participate in this study. Diagnosis was performed by dentists “single examiner”, the subjects were without treatment and with no other chronic or systemic diseases. Apparently healthy volunteers consisted of 30 individuals who were their age range (20-55) years with a mean age of 36.2±6.1 years considered as control. Three ml of whole unstimulated saliva were collected using plastic test tubes. Then saliva centrifuged at 1000 rpm for 10 minutes, the supernatants were aspirated immediately, divided into aliquots and kept at -20°C until used for ACPA assay. RF- IgM was estimated by agglutination test (RF-Spinreact S.A. / Spain), whereas ACCP- IgG, was estimated by using commercially available ELISA and performed as recommended in leaflet with kit (ACCP-Human GmbH. Wiesbaden/ Germany). Statistical analysis: It was assessed using P (Bonferroni-test), Spearman test. P values of P<0.05 were considered significant.

**RESULTS**

The present study was performed on 30 patients with PD and 20 healthy individuals without any periodontal disease. There were 17 males and 13 females in the PD patients, and there were 10 males and 10 females in the healthy individuals group.

Table (1) showed that the mean age of PD patients was 39.1±4.2 years, whereas for healthy subjects was 36.2±6.1 years with no significant differences (p>0.05). The current results showed that there is no significant differences in the presence of RF (p>0.05) between patients and controls, RF-IgM was detected in saliva of 3 patients with PD (10%) and in one case (5%) of 20 healthy control, whereas the salivary levels of ACPA-IgG in chronic PD were significantly elevated as compared to healthy controls (16.29±4.3 RU/ml vs.1.19± 0.9 RU/ml) respectively, (p<0.05), table (2).

**DISCUSSION**

Clinical studies of RA and periodontal disease have provided evidence for a significant association between the two disorders. Patients with PD have a higher prevalence of RA than patients without PD and it may be hypothesized that periodontal disease plays a role as a triggering factor for RA. The current results showed that there is no significant difference in the occurrence of RF between patients and controls, whereas the salivary level of ACPA patients was significantly elevated as compared to healthy controls. In study conducted by The and Ebersole showed that patients with PD who were RF-positive presented elevated Ig-M and IgG against certain periodontopathic species versus RF-negative PD subjects. Moreover, Molitor and colleagues reported that ACCP titters were considerably higher in RA patients with moderate to severe PD than in RA patients without PD. In contrast Mukhtar et al., reported that in RA patients with PD the mean ACCP level was high as compared to that of control and PD groups respectively. But there is no statistically significant differences were observed in ACCP level between PD group and control group.

However, pathogen-specific immunoglobin (IgG and IgA) levels against periodontopathic bacteria have been found to be raised in the synovial fluid of
patients with RA, pointing towards the possibility that these antibodies directed against periodontopathic bacteria could be important in the etiopathogenesis of RA\textsuperscript{13, 14}. The increase in level of salivary ACCP could be attributed to the fact that \textit{P}. \textit{g} is the common oral pathogen strongly involved in the pathogenesis of PD. This bacterium is a gram-negative anaerobic that is recognized to be the only bacterium known to express PAD enzyme which has been identified as a susceptibility factor for RA. Therefore, bacteria play a role in peptide citrullination and involved in loss of self tolerance and development of RA\textsuperscript{15, 16, 17}. These findings indicate that the ACPA detected in the saliva of some periodontitis patients may be elicited by certain micro-organisms in the subgingival plaque. However; our results that supports the hypothesis that oral infections play a role in rheumatoid arthritis pathogenesis, of special importance is the impact of periodontal pathogens, such as \textit{Porphyromonas gingivalis} on citrullination.

Table 1
Age and gender distribution of the studied groups

<table>
<thead>
<tr>
<th>Study groups</th>
<th>PD Patients n=30</th>
<th>Healthy control n=20</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) Range</td>
<td>(25-55)</td>
<td>(20-55)</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>39.1±4.2</td>
<td>36.2±3.1</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17(57%)</td>
<td>13(43%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>10(50%)</td>
<td>10(50%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Chronic PD patients-healthy control difference in salivary levels of RF and ACPA.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>PD Patients n=30</th>
<th>Healthy control n=20</th>
<th>T-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF Positive</td>
<td>3(10%)</td>
<td>1(5%)</td>
<td>p&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>ACCP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(2.7-24.5)</td>
<td>(1.1-4.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>16.29 ± 4.3</td>
<td>1.19± 0.9</td>
<td>p&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES
6. Liao F, Li Z, Wang Y, Shi B, Gong Z, Cheng X. \textit{Porphyromonas gingivalis} may play an important role in the pathogenesis of
periodontitis associated rheumatoid arthritis.


