Evaluation of serum anti-Cardiolipin antibody, hs-CRP and IL-6 levels in chronic periodontitis as possible risk factors for cardiovascular diseases

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ABSTRACT

**Background:** It has long been established that there is an association between cardiovascular disease and periodontitis. Evidence shows periodontally infected patients may be at a higher risk of thrombotic accidents via induced systemic inflammatory mediators' production and increase in serum levels of autoantibodies such as anti-cardiolipin antibody. The aim of the present study was to determine the presence of anti-cardiolipin antibody (ACLA)-IgG and -IgM, and to investigate the systemic levels of inflammatory markers of cardiovascular diseases like high sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6) in patients with chronic periodontitis (CP) as well as to examine the relationships between these mediators and clinical periodontal parameters.

**Subjects and Methods:** Blood samples were collected from 45 patients with CP (20 with severe periodontitis and 25 with moderate periodontitis) and from 30 healthy age matched individuals served as controls. Clinical periodontal parameters used in this study were plaque index, gingival index, probing pocket depth, clinical attachment level and bleeding on probing. The levels of serum ACLA-IgG, ACLA-IgM, hs-CRP and IL-6 were determined using enzyme-linked immunosorbent assays.

**Results:** The current results revealed that serum levels of ACLA-IgG, hs-CRP and IL-6 were significantly higher in patients group as compared to healthy control group (p<0.05, p<0.001), whereas the serum level ACLA-IgM was not observed any significant differences between two groups (p>0.05). Concerning the comparison between two patient groups, severe CP group showed significant elevation in serum levels of ACLA-IgG, -IgM, hs-CRP and IL-6 (p<0.05, p<0.001), while there is no differences in serum level of ACLA-IgM when compared to moderate CP patients group (p>0.05). Furthermore, in regards to the correlation between serum ACLA-IgG, ACLA-IgM, hs-CRP and IL-6, and clinical periodontal parameters, IL-6 level was showed significant positive correlation with clinical attachment level, whereas hs-CRP was showed significant positive correlation with each of probing pocket depth, clinical attachment level and bleeding on probing. Moreover, linear positive correlation was noticed between ACLA-IgG and clinical attachment level. Conversely, ACLA-IgM level did not show any correlation with clinical parameters of periodontitis (p>0.05).

**Conclusion:** Elevation in prothrombotic autoantibodies, ACLA-IgG and inflammatory mediators (hs-CRP and IL-6) factors may increase inflammatory activity in atherosclerotic lesions and potentially increasing the risk for cardiovascular events.

**Key words:** Chronic Periodontitis; Anticardiolipin; hs-CRP, IL-6. (J Bagh Coll Dentistry 2012; 24(Sp. Issue 2):162-165).

INTRODUCTION

Periodontitis is a chronic infectious disease of the supporting tissues of the teeth and it has been consistently associated with cardiovascular diseases (CVD). CVD is the broad term used to categorize any abnormal condition characterized by dysfunction of the heart and blood vessels (1, 2). According to the recent literature it is also possible that the apparent association between these two disease groups is related more to the existence of common risk factors and common underlying physiologies and pathophysiologies (2, 3). Moreover; one explanation in this association is that periodontitis may also cause a prothrombotic state. The prothrombotic state is a propensity of blood to coagulate due to an abnormality in the coagulation and/or fibrinolysis system (4, 5). The systemic dissemination of periodontal pathogens from periodontal lesions seems to be at least one cause for the systemic inflammation in periodontitis and elevation of CVD risk markers.

The periodontal pathogens *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* have been shown in blood and biopsies from atherosclerotic plaques (6, 7). Several parameters of systemic inflammation have been identified, including CRP, IL-1 and IL-6, however; some authors suggest that increase in these systemic markers of inflammation occurs together with increase in serum levels of auto-antibodies including ACLA (8).

Cardiolipin is a phospholipid (diphosphatidylglycerol) found in inner mitochondrial membrane primarily, but it is also a minor constituent of mammalian membranes in general. In diseases with mitochondrial damage cardiolipin can evoke an antibody response (9, 10). Antiphospholipid antibodies are a class of auto-antibodies which have been found in 1-5% of systematically healthy population. These antibodies are also usually detected in patients with systemic lupus erythematosus and antiphospholipids antibody syndrome, in addition are associated with adverse pregnancy outcomes (8). The increased level of these antibodies has also

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been observed in several situations including some infectious diseases, consequently infectious diseases may have a role in production of ACLA, it has also been suggested that patients with periodontitis might have a higher level of ACLA in comparison with periodontally healthy people. Thus, increased ACLA level might explain the association of such systemic disorders as prothrombotic accidents with periodontitis (11).

C-reactive protein is a plasma protein synthesized by the liver and adipocytes, being actually recognized as an important biomarker of a wide spectrum of conditions such as systemic inflammation, infections, neoplasias (lymphoma), and immune-mediated rheumatic disorders including rheumatoid arthritis and vasculitis (12). In addition, hs CRP measures cardiac and cerebrovascular risk being of special interest as risk factor (13). Numerous cytokines have been identified at sites of chronic inflammation such as arthritis and periodontitis. One of these, IL-6 is an important pro-inflammatory cytokine involved in the regulation of host response to tissue injury and infection. It is produced by a variety of cells, such as monocytes, fibroblasts, osteoblasts and vascular endothelial cells in response to inflammatory challenges (14). Moreover, it is widely accepted that IL-6 induces CRP production (15). Since elevated plasma levels of IL-6 have been associated with unstable angina and CVD, IL-6 is actually related to other cardiovascular risk factors (13). Due to the potential association between periodontitis and cardiovascular disease, the current work was carried out to assess ACLA, hs-CRP and IL-6 as possible risk factors for cardiovascular disease in patients with CP, and to determine the relationship of their elevated levels to the severity of periodontal disease.

SUBJECTS AND METHODS

The present study included 45 patients (19 females and 26 males), with an mean of age 42.1±8.071 years, and ranged between (30-55 years), were from attendants seeking treatment in the department of periodontics, College of Dentistry, Baghdad University, during the period between December 2010 till May 2011. Diagnosis was made by specialized dentists (single examiner conducted the periodontal assessment in order to minimize the variation in the data), all the cases had received no treatment with no complain of other chronic or systemic diseases. Compared with 30 age and sex-matched apparently healthy individuals considered as controls. Clinical periodontal parameters used in this study were plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP). CP patients were divided in to two groups based on their clinical attachment loss (CAL), [25 moderate CP with CAL>2mm and 20 sever CP with CAL<5mm].

Clinical attachment level is defined as the distance from the cement enamel junction (CEJ) to the location of the inserted probe tip. The measurements were made at four surfaces of each tooth.

Blood samples was collected from patients and controls to estimated serum concentrations of ACLA-IgG, ACLA-IgM, hs-CRP and IL-6 by using commercially available enzyme-linked immunosorbent assay (ELISA) kits and performed as recommended in leaflet with kits (ACLA-IgG & IgM-AESKU. Diagnostic, Germany; hs-CRP-Calbiotech, Inc USA; IL-6-BioSource Europe S.A. Company, Belgium). Statistical analysis was assessed using P (Mann-Whitney-test), and (Kruskal-Wallis-test). Statistical analysis: It was assessed using P (Mann-Whitney-test), P (Bonferroni-test) and (Kruskal-Wallis-test). Correlation among different parameters was calculated by the spearman test and p values of P<0.01and P<0.05 were considered significant (16).

RESULTS

Forty five Iraqi patients with CP (19 females and 26 males) were recruited for the present study; their mean age was 42.1±8.071 years (range 30-55 years). Demographic results demonstrated that (58%) of patients were males and (42%) were females. The differences in clinical periodontal parameters between patients and healthy controls are summarized in table (1).

The current results revealed that mean serum levels of ACLA-IgG, hs-CRP and median serum level of IL-6 were significantly higher (p<0.05, p<0.001) in patients group (8.22±7.27 iu/ml, 1.86±1.51 mg/dl and 5.2 pg/ml respectively) as compared to healthy control group (6.11±3.83 iu/ml, 0.68±0.44 mg/dl and 2.15 pg/ml respectively), whereas the serum level ACLA-IgM was not observed any significant differences (p>0.05) between two groups as clearly shown in table (2). Concerning the comparison between two patient groups (sever and moderate CP), sever CP group showed significant elevation (p<0.05, p<0.001) in serum levels of ACLA-IgG, hs-CRP and IL-6 (12.13±8.48 iu/ml, 2.53±1.69 mg/dl and 6.5 pg/ml respectively), while there is no significant differences (p>0.05) in serum level of ACLA-IgM when compared to moderate CP patients group, table (3). Furthermore, in regard to
the correlation between serum ACLA-IgG, ACLA-IgM, hs-CRP and IL-6, and clinical periodontal parameters, linear positive correlation was noticed between ACLA-IgG and CAL $(r=0.434, P=0.030)$. Conversely, ACLA-IgM level did not show any correlation with clinical parameters of periodontitis $(P>0.05)$. Interestingly, hs-CRP was showed significant positive correlation with each of PPD, CAL and BOP $(r=0.389, P=0.049; r=0.444, P=0.026; r=0.579, P=0.002$ respectively), whereas IL-6 level was revealed significant positive correlation with CAL $(r=0.466; P=0.019)$ as observed in table (4).

DISCUSSION

Periodontitis is very common and is regarded as the second most common disease worldwide, after dental decay. Over the past two decades, there has been an increasing interest in the possible link between dental disease, specifically periodontal disease, and CVD (1). Inflammation plays an important role in the pathogenesis of atherosclerosis, and markers of low grade inflammation have been consistently associated with a higher risk of CVD. It has been observed that people with periodontal disease are at a greater risk of systemic diseases such as CVD (17).

The present work is found increase in serum levels of ACLA-IgG in CP patients when compared to controls which is in accordance with the observations of the previous researchers (18-20); Schenkein et al (18), evaluated serum ACLA level in patients with severe periodontitis, and suggested that increase in systemic markers of vascular endothelial inflammation occurs together with increase in level of serum ACLA. Consistency Faghihi and colleagues observed that the mean serum ACLA level of patient group was significantly higher than that of the control group although all cases had a normal range of ACLA (19), on the other hand, other study conducted by Sumanth et al. (21) denoted that serum ACLA-IgM and IgG levels were significantly higher in patients with acute myocardial infarction associated with CP than in patients with acute myocardial infarction. In addition, they showed significant alterations in concentrations of serum ACLA-IgM and IgG levels after phase I periodontal therapy. Interestingly, the present study failed to show any significant differences in serum ACLA-IgM levels between patients and control groups as well as between severe and moderate CP, this result was at variance with some other studies (20, 21), who found significant increase in serum ACLA-IgM level in severe CP patients when compared to healthy individuals. Correspondingly to our results Türkoğlu et al (22) found a positive correlation between ACLA-IgG levels and CAL, so they conclude that CP might be associated with an increased level of serum ACLA.

The possible explanation for the higher levels of serum ACLA-IgG may be due to the fact that since infectious diseases may induce the production of ACLA, it can be suggested that patients with periodontitis may show an increased level of serum ACLA. This increase might explain the presence of systemic disorders including prothrombic accidents (such as stroke) and fetal abortion in periodontitis patients (23). Several inflammatory biomarkers have already been validated as cardiovascular risk factors, particularly CRP, an emerging and reliable biomarker of the acute phase response to infectious burdens and/or inflammation. In addition, IL-6 may also be listed among factors contributing to the association between chronic infections and CVD, displaying pro-inflammatory and pro-coagulant properties (26).

Other important findings in this study were the significant elevation of mean serum level of hs-CRP and median serum level of IL-6 in patients with periodontitis, particularly in the subgroup of patients with severe CP. These results are comparable to other previous results reported by Gani et al. (25), Fitzsimmons et al. (26), and Haba et al. (27). More recent evidences, however, has indicated that patients with severe periodontitis have increased serum levels of CRP, hyperfibrinogenemia, moderate leukocytosis, as well as increased serum levels IL-6 when compared with unaffected control populations (27). In contrast, Ido and co-workers reported that there were no statistically significant changes in the levels of any of the aforementioned systemic markers. They concluded that improvement in periodontal health also did not influence the levels of vascular markers (28). However, different results in various studies may be due to the case selection, the volume of inflammatory tissues, or the methods used. Certainly, more studies taking into account other variables are required in this field.

Regarding the correlation between serum (hs-CRP and IL-6) and clinical periodontal parameters, hs-CRP was showed significant positive correlation with each of PPD, CAL and BOP, whereas IL-6 level was revealed significant positive correlation with CAL. The results obtained from the present study were similar to that reported by others investigators (26, 27, 28).

The elevated levels of CRP and IL-6 in periodontitis patients may occur when bacteria and bacterial products, such as...
lipopolysaccharide, as well as locally produced pro-inflammatory cytokines enter the circulation. CRP and IL-6 may contribute, in part, to the observed associations between chronic infections and CVD. CRP may activate complement in damaged vessel walls whereas IL-6 has pro-inflammatory properties and a pro-coagulant effect. These properties may contribute to the pathogenesis of coronary syndromes. Furthermore, IL-6 stimulates the production of CRP by hepatocytes.

Finally, Azarpazhooh and Tenenbaum (30) reported that several studies showed a weak but statistically significant association between CVD and periodontal disease. Although the risk estimates might be considered modest, the high prevalence of both types of disease means that the absolute numbers of those affected is quite high. Hence, they reported that an individual with periodontitis is at greater risk of either having or developing CVD. In conclusion current results suggest that elevation in prothrombotic mediators (hs-CRP and IL-6) factors may increase inflammatory activity in atherosclerotic lesions and potentially increasing the risk for cardiovascular events in sever CP patients.

REFERENCES


**Table 1: Demographic and Clinical Parameters in Patients and healthy Control groups**

<table>
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<tr>
<th>Demographic Parameters</th>
<th>Patients (n=45)</th>
<th>Healthy Control (n=30)</th>
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<td>Age Mean ± SD</td>
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<td>36.6±5.567</td>
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<tr>
<td>Male</td>
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<td></td>
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**Clinical Parameters**

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<thead>
<tr>
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<th>GI</th>
<th>PD</th>
<th>AL</th>
<th>BOP</th>
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<td>Pat.</td>
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<td>1.331</td>
<td>2.455</td>
<td>1.851</td>
<td>28.62</td>
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<tr>
<td>Pat.</td>
<td>0.613</td>
<td>0.53</td>
<td>1.126</td>
<td>0</td>
<td>4.766</td>
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</table>

*P<0.05 Significant, **P<0.001 High significant

**Table 2: Patients and healthy control differences in serum concentration of ACLA-IgG, ACLA-IgM, hs-CRP and IL-6**

<table>
<thead>
<tr>
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<td>ACLA-IgG</td>
<td>23.8</td>
<td>17.1</td>
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<td>ACLA-IgM</td>
<td>51.9</td>
<td>18.9</td>
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<td>4.75</td>
<td>9.36</td>
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<td>2</td>
<td>1.2</td>
<td>0.6</td>
<td>1.86</td>
<td>0.68</td>
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<tr>
<td>IL-6</td>
<td>10.6</td>
<td>8.6</td>
<td>5.2</td>
<td>2.15</td>
<td>7.3</td>
<td>3.64</td>
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**Table 3: Sever CP and moderate CP differences in serum concentration of ACLA-IgG, ACLA-IgM, hs-CRP and IL-6**

<table>
<thead>
<tr>
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<td>ACLA-IgG</td>
<td>23.8</td>
<td>18</td>
<td>10.95</td>
<td>4.5</td>
<td>12.13</td>
<td>5.052</td>
<td>8.48</td>
<td>4.11</td>
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<td>18.9</td>
<td>7</td>
<td>5.8</td>
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<td>3.1</td>
<td>2.9</td>
<td>0.9</td>
<td>2.531</td>
<td>1.324</td>
<td>1.69</td>
<td>1.11</td>
<td>0.005</td>
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<tr>
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<td>6.6</td>
<td>6.5</td>
<td>3.4</td>
<td>5.545</td>
<td>3.304</td>
<td>2.96</td>
<td>2.32</td>
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**Table 4: Correlation between serum concentration of ACLA-IgG, ACLA-IgM, hs-CRP and IL-6 and clinical periodontal parameters in CP cases**

<table>
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<th>Clinical Parameters</th>
<th>PI Correlation (Mann-Whitney)</th>
<th>P</th>
<th>GI Correlation (Mann-Whitney)</th>
<th>P</th>
<th>CAL Correlation (Mann-Whitney)</th>
<th>P</th>
<th>PPD Correlation (Mann-Whitney)</th>
<th>P</th>
<th>BOP Correlation (Mann-Whitney)</th>
<th>P</th>
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<td>-0.054</td>
<td>0.797</td>
<td>-0.239</td>
<td>0.257</td>
<td>0.434</td>
<td>0.030</td>
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<td>0.731</td>
<td>-0.306</td>
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<tr>
<td>ACLA-IgM</td>
<td>0.000</td>
<td>0.998</td>
<td>0.142</td>
<td>0.498</td>
<td>-0.149</td>
<td>0.479</td>
<td>-0.114</td>
<td>0.588</td>
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<td>0.548</td>
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<tr>
<td>hs-CRP</td>
<td>0.095</td>
<td>0.651</td>
<td>-0.105</td>
<td>0.616</td>
<td>0.389</td>
<td>0.049</td>
<td>0.444</td>
<td>0.026</td>
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<td>0.004</td>
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