Evaluation of Serum Anti-Cardiolipin Antibody Titer in Patients with Chronic Periodontitis

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Abstract:
Objective: Evidence shows periodontally infected patients may be at a higher risk of thrombotic accidents and adverse pregnancy outcomes, via induced systemic inflammatory mediators’ production. Some authors have concluded that increase in systemic inflammatory markers occurs together with increase in serum levels of auto antibodies including anti-cardiolipin antibody (ACLA). The aim of the present study was to compare the serum ACLA level between patients with chronic periodontitis (CP) and periodontally healthy controls.

Materials and Methods: Fifty-one patients with moderate and advanced CP (test group) and 49 periodontally healthy people (control group) were included in the study. Clinical parameters including PI, GBI, PPD and CAL were measured. Serum ACLA level of all cases was measured using ELISA method. The data were analyzed with Student t-test and Pearson's correlation.

Results: A significant difference existed in serum ACLA level between test and control groups (P=0.001). All cases in both test and control groups, however, showed a normal range of serum ACLA level.

A positive correlation also existed between serum ACLA level and periodontal parameters including CAL, PPD, GBI and PI (P<0.001, P<.001, P=0.001 and P=0.002, respectively). In addition, a moderately positive correlation (P=0.003) between age and ACLA level was found.

Conclusion: An increased serum ACLA level might be associated with chronic periodontitis.

Key Words: Chronic Periodontitis; Cardiolipins; Antibodies, Anticardiolipin; Autoantibodies

INTRODUCTION
Recent evidence suggests that periodontally infected patients may be at a higher risk of systemic disorders including cardiovascular diseases and delivery of preterm or low-birthweight infants [1].

It is suggested that association of these conditions with periodontitis results from the effect of periodontal pathogens especially gram negative bacteria, which induce the production of systemic inflammatory mediators [2-5]. Some authors suggest that increase in systemic markers of vascular endothelial inflammation occurs together with increase in serum levels of auto antibodies including anti-cardiolipin antibody (ACLA) [2]. Accordingly, the association between periodontitis and increased serum levels of ACLA has recently been taken...
Cardiolipin is a phospholipid (diphosphatidylglycerol) found in inner mitochondrial membrane primarily, but it is also a minor constituent of mammalian membranes in general [6]. In diseases with mitochondrial damage cardiolipin can start an antibody response [7]. Anti-phospholipid antibodies are a class of autoantibodies which have been found in 1-5% of systematically healthy population [8]. These antibodies are also usually detected in patients with systemic lupus erythematosus (SLE) and anti-phospholipids antibody syndrome (APLS) [2,9]. The increased level of these antibodies has also been observed in several situations including some infectious diseases, and has been recognized as a sign of APLS as well [2,10]. In this vein, some recent evidence has shown that bacterial and viral infections have a role in etiology of APLS via induction of ACLA production [11]. It should be mentioned that APLS patients have a tendency to thrombosis but the mechanism is still not transparent [12].

On the other hand, a similarity exists between symptoms of APLS and attributed systemic consequences of periodontal infection such as prothrombotic accidents, adverse pregnancy outcomes and fetal abortions. While 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 [2,10]. Accordingly, Taylor et al [13] reported that elimination of periodontitis leads to a decrease in thrombotic and inflammatory markers, which are risk factors for cardiovascular diseases.

The aim of the present study was to compare the serum ACLA level between patients with chronic periodontitis (CP) and periodontally healthy controls.

### MATERIALS AND METHODS

The study sample comprised fifty-one chronic periodontitis patients (24 females and 27 males) as the test group and 49 periodontally healthy people (20 females and 27 males) as control group. The samples were selected among people referring to periodontics and oral medicine departments in Dental School of Shiraz University of Medical Sciences, who were volunteers to participate in the study. A check list through which medical and dental history were recorded, was completed for all participants. The patients who had a history of diabetes, infection, taking antibiotics during the past three months, periodontal therapy during the past two years, hepatitis and autoim-

### Table 1. ACLA titer and clinical periodontal parameters in patients with chronic periodontitis (test group, n=51) and health controls (n=49).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Difference</th>
<th>SD</th>
<th>P-value</th>
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<td>1.41</td>
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<td>0.001</td>
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<td>2.37</td>
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<td></td>
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<td>5.13</td>
<td>1.34</td>
<td></td>
<td></td>
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<tr>
<td>PPD</td>
<td>Control</td>
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<td>2.29</td>
<td>0.16</td>
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<td>0.91</td>
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<tr>
<td>GBI</td>
<td>Control</td>
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<td>3.78</td>
<td>49.38</td>
<td>2.17</td>
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<td></td>
<td>Test</td>
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<td>54.02</td>
<td>14.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Control</td>
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<td>44.18</td>
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<td>57.46</td>
<td>13.48</td>
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</table>

N=Number of teeth, SD=Standard Deviation, ACLA=Anti-Cardiolipin Antibody (unit/ml), CAL=Clinical Attachment Level, PPD=Periodontal Pocket Depth, GBI=Gingival Bleeding Index (%), PI=Plaque Index (%).
immune diseases, and smoking, in addition to pregnant and nursing women were excluded from the study. The cases had at least 20 teeth in their mouth and they did not have any history of periodontal surgery. The participants signed an informed consent form approved by local ethics committee.

Periodontal examination comprised measuring periodontal pocket depth (PPD) and clinical attachment level (CAL) in millimeters with a William's periodontal probe, recording plaque control (PI) by O'Leary plaque index [14], and recording gingival bleeding index (GBI) [15]. The parameter of GBI was used to determine whether or not periodontal inflammation is present, and PI was measured to clarify the consistency of local factors with periodontal attachment loss. CAL is the clinical parameter to detect the presence of periodontal attachment loss and determine the severity of periodontitis [16].

Patients in CP (case) group had moderate (patients in any age with 3-4 mm of attachment loss in more than 30% of sites with any degree of severity which was consistent with local factors) or advanced (patients in any age with ≥5 mm of attachment loss in more than 30% of sites with any degree of severity which was consistent with local factors) chronic periodontitis. Samples with normal periodontium (NP) as the control group were those with no sign of attachment loss, and the sulcular depths were ≤3 mm. They also did not have any site of gingival recession to be a sign of periodontitis and did not take any periodontal treatment previously [16].

Laboratory process: A 5cc blood sample was taken in laboratory from all participants. It was stored at -30°C until utilized and ELISA test was done to determine ACLA level in serum. ELISA test measured the quantitative level of IgG, IgM and IgA class auto-antibodies against cardiolipin human plasma or serum.

Highly purified cardiolipin binds to β2-Glycoprotein-I (β2 GP I) in microwells filled with β2GP-I. Antibodies against these antigens bind to the antigen if it is present in diluted plasma or serum. Horseradish peroxidase (HRP) conjugates IgG, IgM and IgA and can detect bound antibodies to antigen to make a conjugated antigen-antibody complex. Microwell washing was done to eliminate nonspecific components and unbound conjugates.

An enzyme substrate hydrolyzes in the presence of conjugated complex and makes a blue color. Adding an acid stop leads to a final production, which is yellow in color. The intensity of the yellow color is measured with photometry (450 nm). According to the kit manufacturer Anti-CL level <20 u/ml was considered as a normal range.

The data were analyzed with Student t-test and Pearson's correlation.

RESULTS

From 51 patients (test group), 24 were females and 27 were males, with age ranging from 30 to 54 years with a mean of 41.17 (SD=6.68). The control group comprising 20 females and 29 males ranged from 30 to 55 years old with

<table>
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<th>Mean</th>
<th>Pearson’s correlation (r)</th>
<th>P-value</th>
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<td>0.000*</td>
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<tr>
<td>GBI</td>
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<td>0.280</td>
<td>0.002*</td>
</tr>
<tr>
<td>PI</td>
<td>35.81</td>
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<td>0.001*</td>
</tr>
<tr>
<td>Age</td>
<td>39.24</td>
<td>0.267</td>
<td>0.006*</td>
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</table>

ACLA=Anti-Cardiolipin Antibody, CAL=Clinical Attachment Level, PPD=Periodontal Pocket Depth, GBI=Gingival Bleeding Index (%), PI=Plaque Index, *= Significant
mean of 37.22 (SD=6.22).

Table 1 shows descriptive data relevant to the mean of ACLA titer and CAL, PPD, GBI, PI for the two groups, and the difference in ACLA titer between them.

A significant difference existed in ACLA titer between test and control groups (P=0.001). All the considered clinical parameters (CAL, PPD, PI and GBI) in test group were higher than those of control group significantly (P<0.001). ACLA titer was also significantly increased in test group (Table1).

When we considered the two genders separately, the difference of ACLA titer between test and control groups in women was significant (P=0.001), but in men was not significant (P=0.200). The difference of PI, GBI and PPD between test and control groups, however, was the same in men and women (P<0.001). CAL was also significantly higher in test group compared to control group among both men and women ((P=0.001 and P<0.001, respectively).

A significant positive correlation was found between ACLA titer and CAL and PPD (P<0.001) (Table 2). A moderately significant positive correlation also existed between ACLA titer and GBI, PI and age (P=0.002, P=0.001, and P= 0.006, respectively).

**DISCUSSION**

In the present study, serum ACLA level was measured in 51 patients with chronic moderate and severe periodontitis (test group) and 49 periodontally healthy people (control group) to assess the assumed association between chronic periodontitis and increased serum level of ACLA.

In our study, the mean serum ACLA level of test group was significantly higher than that of the control group although all cases had a normal range of ACLA according to the kit manufacturer.

It should be mentioned that auto-antibodies which have a high affinity to anionic phospholipids, are usually reported to be associated with thrombosis, thrombocytopenia and frequent fetal abortion known as APLS. Although APLS occurs in SLE and other autoimmune diseases, it might also occur in some infectious diseases [11,17].

On the other hand, as mentioned previously, some common symptoms exist between APLS and systemic consequences attributed to periodontal infections. 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 [2]. Accordingly, Fenner et al [18] in reporting two cases of fatal thrombotic thrombocytopenic purpura (TTP) recommended that to prevent recurrence of TTP, periodontally questionable teeth should be extracted. Several studies, for example Schenkein et al [19], evaluated serum ACLA level in patients with generalized aggressive periodontitis. They came to the conclusion that increase in systemic markers of vascular endothelial inflammation occurs together with increase in level of serum ACLA [2,19].

The results can be in corroboration with our study. However, we cannot compare our results conclusively with the above findings because our participants in test group suffered from chronic periodontitis (CP). Furthermore, Schenkein et al found increased vascular inflammatory markers in patients who had ACLA level beyond normal range (>15 u/ml), or patients with positive ACLA test. In our study, higher serum ACLA level in patients with chronic periodontitis than that in healthy controls lied in the normal range according to our laboratory kit manufacturer. Thus, whether elevating serum ACLA levels (within the normal range) in our test group could be associated with increasing endothelial inflammation markers, remains to be seen. Furthermore, in Schenkein
et al study [2], ACLA level was positively correlated with presence of generalized chronic and aggressive periodontitis, but not with localized aggressive periodontitis. We did not divide periodontitis to generalized and localized forms in our study, and our test group suffered from generalized CP.

Another finding reported by Schenkein et al [2] in localized and generalized aggressive periodontitis suggests a role for volume of inflammatory tissue or age in increasing ACLA levels. In this vein, we also found that age had a significant positive but weak correlation with ACLA level.

On the other hand, our results can be consistent with those of Türkoğlu et al [20] who found increased levels of serum ACLA in chronic periodontitis patients suffering from essential blood pressure. They suggested that increasing ACLA levels could increase the risk of atherosclerosis in these patients [20], as other authors have found a possible association between chronic periodontitis and increased incidence of atherosclerotic complications [21].

Similar to our results, Türkoğlu et al [20] found a positive correlation between ACLA levels and supragingival plaque, GBI, PD and CAL. Nonetheless, some differences existed between our methods and those of Türkoğlu et al. For example, in our case selection we excluded patients with systemic disorders that could interfere with our results. Furthermore, they measured IgM and IgG ACLA separately while we assessed the total antibody titer. ACLA is found in IgM and IgG classes [22], but similar to some other studies we measured only total antibody titer.

**CONCLUSION**

Chronic periodontitis might be associated with an increased level of serum ACLA. 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.

**REFERENCES**


