#### **Original Article**

# **Evaluation of Serum Anti-Cardiolipin Antibody Titer of** Patients with Chronic Periodontitis in Comparison with **Periodontally Healthy Population**

# SH. Faghihi<sup>1</sup>, AR. Rokn<sup>≤2</sup>\*, R. Ebrahimi<sup>3</sup>

<sup>1</sup>Assistant Professor, Department of Periodontics, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran <sup>2</sup>Associate Professor, Department of Periodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran <sup>3</sup>Postgraduate Student, Department of Periodontics, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

#### Abstract:

	Objective: Evidence shows periodontally infected patients may be at a higher risk of
	thrombotic accidents and adverse pregnancy outcomes, via inducing systemic inflamma-
	tory mediators' production. Some authors have concluded that increasing systemic in-
	flammatory markers occurs together with increased serum levels of autoantibodies includ-
	ing anti-cardiolipin antibody (ACLA). Therefore, the aim of this study was to compare the
	serum ACLA level between patients with chronic periodontitis (CP) and those who are
	periodontally healthy.
	Materials and Methods: 51 Moderate and advanced CP patients (test group) and 49 pe-
	riodontally healthy people (Control group) were included in the study. Clinical parameters
	including PI, BOP, PPD, and CAL were measured. Serum ACLA level of all cases was
	measured by ELISA. The data were analyzed with t-test and Pearson's correlation.
	<b>Results:</b> There was a significant difference in serum ACLA level between test and control
	groups (P=0.001). Although all cases in both test and control groups showed a normal
	range of serum ACLA level.
	There was also a positive correlation between serum ACLA level and periodontal parame-
	ters including CAL, PPD, BOP and PI (P<0.001, <0.001, =0.001 and =0.002 respectively).
	In addition, a moderately positive correlation (P=0.003) between age and ACLA level was
	obtained.
AR Rokn Department of Perio-	Conclusion: An increased serum ACLA level might be associated with chronic periodon-
dontics, School of Dentistry,	titis.
Tehran University of Medical	
Sciences, Tehran, Iran.	Key Words: Chronic periodontitis, cardiolipin, anti-cardiolipin antibodies, auto antibod-
roknamir@sina.tums.ac.ir	ies

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#### **INTRODUCTION**

There is some recent evidence that shows periodontally infected patients may be at a higher risk of systemic disorders including cardiovascular diseases and preterm birth or low birth weight of infants [1].

It is suggested that the association between periodontitis and the afore-mentioned disorders is related to causal effect of periodontal

pathogens especially Gram-negative bacteria, which induce the production of systemic inflammatory mediators [2-5].

Some authors suggest that increase of systemic markers of vascular endothelial inflammation occurs together with increased serum levels of autoantibodies including anti-cardiolipin antibody (ACLA) [2]. Accordingly, the association between periodontitis and increased serum

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levels of ACLA has recently been taken into account.

Cardiolipin can start an antibody response in diseases with mitochondrial damage [6]. Cardiolipin is a phospholipid (diphosphatidylglycerol) found in inner membrane of mitocondries primarily, but it is also a very minor constituent of mammalian membranes in general [7].

Anti- phospholipid antibodies are a class of autoantibodies, which have been found in 1-5% of systematically healthy population [8]. In addition, these antibodies are usually detected in patients with systemic lupus erythematous (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 accounted 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, there is a similarity 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 periodntitis might have a higher level of ACLA in comparison with periodontally healthy people. So, increasing ACLA level might explain the association between systemic disorders like prothrombotic accidents and periodontitis [2,10]. Accordingly, taylor, et al reported that elimination of periodontitis leads to a decrease of thrombotic and inflammatory markers which are risk factors for cardiovascular diseases [13].

Since some evidence shows that periodontal infections might induce the production of ACLA which may be a risk factor for cardiovascular diseases, the aim of this study is to compare the serum ACLA level in patients with chronic periodontitis and that of periodontally healthy population.

## MATERIALS AND METHODS

51 chronic periodontitis patients (24 females and 29 males) as the test group and 49 periodontally healthy people (20 females and 29 males) as control group were included in the study.

They were selected among people referring to periodontics and oral medicine departments in Dental School of Shiraz University of Medical Sciences, through volunteers participating 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 autoimmune diseases, smoking and also pregnant and nursing women were eliminated 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 included 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 bleeding points on probing (BOP%) by a simplified bleeding index [15].

The parameter of BOP was used to determine whether periodontal inflammation is present or not 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 if so, determine the severity of periodontitis [16].

The cases in this study were divided into two

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#### groups:

1) Chronic periodontitis (CP)

-Moderate chronic peridontitis; 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.

-Advanced chronic peridontitis; patients in any age with  $\geq 5$  mm of attachment loss in more than 30% of sites with any degree of severity, which was consistent with local factors.

2) People with normal periodontium (NP) as the control group who had no sign of attachment loss and the sulcular depths were  $\leq 3$  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 [17].

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

Highly purified cardiolipin binds to  $\beta$ 2-Glycoprotein-I ( $\beta$ 2 GP I) in microwells filled with  $\beta$ 2GP-I. Antibodies against these antigens bind to the antigen if it is present in diluted plasma or serum.

Nonspecific components were removed by washing microwells. Horseradish perioxidase (HRP) conjugates IgG, IgM and IgA and can detect bound antibodies to antigen to make a conjugated antigen-antibody complex.

Microwell washing eliminates 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 statistical analyses conducted, comprised of a 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 including 20 females and 29 males ranged from 30 to 55 years old with mean of 37.22 (SD=6.22).

Descriptive data relevant to the mean of ACLA titer and CAL, PPD, BOP, PI for the two groups were recorded in table1. The difference of ACLA titer between control and test groups is also included in this table.

There was a significant difference in ACLA titer between control and test groups (P=0.001). The difference in CAL, PPD, PI and BOP between the two groups was also significant (P<.001). All the clinical parameters in test group were higher than that of control group significantly. ACLA titer was significantly increased in test group (table1).

When we considered gender, in women the difference of ACLA titer between test and control groups was significant (P=.001), but in men the difference was not significant (P=.200). Considering gender, the difference of PI, BOP and PPD between test and control groups was the same in men and women (P<0.001). There was also a significant difference of CAL between test and control groups both in men and women ((P=0.001), P<.001, respectively).

Table 2, shows that there was a significant positive correlation between ACLA titer and increasing CAL and PPD (P<0.001). There was also a moderately significant positive correlation between ACLA titer and Bop, PI and age (P=0.002, 0.001, 0.006, respectively).

# DISCUSSION

In this study, serum ACLA level was measured in 51 patients with chronic moderate and severe periodontitis (test group) and 49 peridontally 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 (P=.001) although all cases had a normal range of ACLA according to the kit manufacturer.

It should be mentioned that autoantibodies 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 occur in infectious diseases in the absence of autoimmune disorders [11,18 ].

On the other hand, there are some common symptoms 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 in 2 cases of fatal thrombotic thrombocytopenic purpura (TTP) recommended that to prevent recurrence of TTP, periodontally questionable teeth be extracted [19].

Several studies like Schenkein's et al evaluated serum ACLA level in patients with generalized aggressive periodontitis. They came to the conclusion that increase of systemic markers of vascular endothelial inflammation occurs together with increased level of serum ACLA [2, 20].

Although the results can be in corroboration with our study, we cannot compare our results conclusively with the above findings. Because, our participant test group suffered from chronic periodntitis (CP). Furthermore, in Schenkein's et al study, they found increased vascular inflammatory markers in patients who had elevated ACLA level more than normal range (>15 u/ml) called positive ACLA test.

In our study, in spite of higher serum ACLA level in patients with chronic periodontitis than that of healthy group, the test group did not show ACLA level more than the normal range (according to our laboratory kit manufacturer). Therefore, whether only elevating serum ACLA level in CP patients could be associated with increasing markers of endothelial inflammation or not, remains to be seen.

Furthermore, in Schenkein's et al study [2], they found a positive correlation between generalized chronic periodontitis, generalized aggressive periodontitis, and ACLA level. But the relation was not found in that of localized aggressive periodontitis. In our study, we did not divide periodontitis to generalized and localized forms, and our test group suffered from generalized CP.

From different Schenkein's results in localized and generalized aggressive periodontitis, it can be assumed the volume of inflammatory tissue or age might have a role in increasing ACLA level. In this vein, we also found that age had a significant positive but low correlation with ACLA level.

On the other hand, our results can be consistent with those of Turkoglu's et al (2008) who found increased levels of serum ACLA in chronic periodontitis patients suffering from essential blood pressure. They suggested that increasing ACLA level can increase the risk of atherosclerosis in these patients [21], as other authors have found a possible association between chronic periodontitis and increased incidence of atherosclerotic complications [22].

Turkoglu et al found a positive correlation between ACLA levels and supragingival plaque, Bop, PD, CAL, and our results confirmed the same findings. Nonetheless, there were some differences between our case selection and methods and those of turkoglu's. For example, in our study for case selection, systemic disorders that could interfere with our results were

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excluded. Furthermore, they measured IgM and IgG ACLA separately while others assessed the total antibody titer. ACLA is found in IgM and IgG classes [23], but 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. Of course, more studies are required in this field by taking into account other variables.

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