Original Article

# Evaluation of anticardiolipin antibodies in tobacco users and non-tobacco users with severe chronic periodontal disease

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## Abstract

**Aims:** Many studies have proven that b2-glycoprotein-I-dependent anticardiolipin is elevated in periodontal diseases. Systemic lupus erythematosus and antiphospholipid syndrome, which are usually associated with high antiphospholipid antibodies, are more prone to adverse pregnancy outcomes and cardiovascular sequelae. Therefore, the aim of the present study is to evaluate IgG, IgM anticardiolipin antibodies in tobacco users and non-tobacco users with severe chronic periodontal disease. **Materials and Methods:** Based on the Armitage classification, 2000, 40 severe periodontitis (group D) (mean clinical attachment loss greater than 2.5 mm) male patients were selected for the study with the age range of 35–65 years and good general health from the Department of periodontics, SRM Kattankulathur Dental College, Chennai. They were classified as smokers (20 subjects) and non-smokers (20 subjects). Blood samples were collected and IgG, IgM antibodies were semi-quantitatively analyzed by enzyme-linked immunosorbent assay. The data thus collected were statistically analyzed by independent student's *t*-test. **Results:** Results showed that smokers with severe periodontitis exhibited marked increase in anticardiolipin IgG, IgM compared to non-smokers. They showed a positive correlation and statistical significance ($P < 0.0001$) between mean clinical attachment loss and IgG and IgM values. **Conclusions:** Results showed a rise in anticardiolipin antibodies in smokers with severe periodontitis, which indicates that these patients are more prone to coronary heart disease.

**Key words:** *Anticardiolipin antibodies, coronary heart disease, IgG, IgM, periodontitis, smoking*

## INTRODUCTION

In the past few years, there has been increasing evidence for the association between periodontitis and cardiovascular diseases. Periodontitis is a chronic inflammatory disease and progresses by bacterial colonization around the tooth, which stimulates immunomodulatory response affecting the periodontium, resulting in bone and tooth loss.[1-3] Periodontal disease could be a risk factor for the development of angina which has become a major medical problem, with increased mortality rate among the Indian population.[3-6] Smoking is one of the significant risk factors for periodontitis affecting the prevalence, extent, and severity of the disease.[7]

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Few studies have proven that 1–5% of systemically healthy population can show elevated levels of antiphospholipid antibodies (APA).[8] In several infectious conditions, increased levels of these antibodies are seen. These APAs and its prothrombotic activity are the major hallmark of the pathogenesis of antiphospholipid syndrome (APS).[9]

Anticardiolipin antibody (aCLA) is the primary antibody commonly seen in patients with APS directed against b2-glycoprotein-I-dependent (b2GPI) phospholipid. This phospholipid plays an important role in platelet aggregation and cell death.[10,11] When b2GPI binds to cardiolipin, an anionic lipid present in nucleated cell membranes and inner mitochondrial membranes can be recognized by aCLA.[12,13]

Smoking is an independent major risk factor for periodontal disease and coronary heart disease.Recent studies show that smoking can increase aCLAs in systemic lupus erythematosus predominant patients.[14] The aim of the current study is to evaluate the presence of aCLAs in tobacco users and non-tobacco users with severe chronic periodontal disease.

### Aims and objectives

Quantitative analysis of IgG, IgM aCLAs in smokers and non-smokers with severe chronic periodontitis.

## SUBJECTS AND METHODS

Based on the Armitage classification, 2000,[15] 40 severe periodontitis (mean clinical attachment loss greater than 2.5 mm) male patients were selected for the study, with the age range of 35–65 years and good general health, who visited the Department of Periodontology SRM Kattankulathur Dental College, Chennai. This study was approved by the SRM university ethical committee. Duration of the study was approximately 4 months (July 2015 to November 2015). All protocols were followed according to the Helsinki declaration. The participants were classified in to smokers (20 subjects) and non-smokers (20 subjects). Study samples were selected based on Statistician's recommendations (for 95% confidence interval, $P < 0.01$).

### Inclusion criteria (for both groups)

Age 35 to 65 years; only males: smokers, those who have smoked more than 100 cigarettes in their lifetime and are currently smoking,[7] and non-smokers.

### Exclusion criteria

Alcohol consumption, malignancy, autoimmune disorders, diabetes, myocardial infarction, hypertension, stroke.

### Patient consent

Patients were informed orally about the procedure, and those who agreed, participated in the study by signing the consent form.

### Study design

Patients included in the study were screened by single periodontist using a mouth mirror and William's periodontal probe using direct and indirect illumination in both groups. All patients underwent periodontal evaluation and hematological and biochemical analysis. All subjects provided informed consent for use of their samples. Clinical periodontal parameters of probing depth and clinical attachment level were calculated.

### Clinical parameters

Probing depth: Probing depth was measured from the gingival margin to the base of the pocket using a calibrated a William's periodontal probe.

Clinical attachment level: Clinical attachment level was measured from the cementoenamel junction to the base of the pocket using a calibrated William's periodontal probe.

All 40 participants showed mean clinical attachment loss more than 2.5 mm (Armitage classification.[15]

### Sampling of blood

2 ml of venous blood sample was obtained by venepunture of the cubital vein in the ante cubical fossa using a 2 ml sterile disposable syringe with a 23 gauge needle. The blood was then transferred to an empty sterile vacutainer and then transported to the clinical laboratory for analysis of aCLA IgG, IgM.[16]

### Estimation of anticardiolipin antibodies ELISA kit Varelisa reagents/material standardization

ELISA kits from Sweden Diagnostics kit, Varelisa IgM Cardiolipin Antibodies, and Varelisa β2-Glycoprotein 1 (IgG) Antibodies were used to asses IgG and IgM aCL and IgG anti-β2GPI. As per the manufacturer's instructions, positive results were considered if the test result was greater than 15 units/mL.[17]

## RESULTS

Data were analyzed by independent student's *t*-test. Results of the current study showed that smokers [Table 1] with severe periodontitis exhibited marked increase in aCLA IgG, IgM compared to non-smokers. The results were statistically significant ($P < 0.001$) in smokers in severe periodontitis subjects when compared to non-smokers.

Results were analysed using independent student's *t*-test [Table 2]. It was noted that IgG, IgM levels were increased in smokers compared to non-smokers. IgG levels were significantly increased (mean difference of 9.14000) compared to IgM levels (mean difference of 8.52050) in severe smokers. Increase in IgG levels show that inflammatory by-products due to smoking were consistently high corresponded to duration.

## DISCUSSION

The principal new finding in this study was that severe chronic periodontitis patients showed elevated IgG, IgM anticardiolipin levels in smokers compared to non-smokers. Smokers are prone to arterial thrombosis and periodontitis. These results indicate that smokers would be at great risk for systemic heart diseases and brain strokes.

Previous studies[18] have shown that IgG in patients with periodontitis, who also have elevated serum concentrations of aCLA, stimulates increased production of the key cytokine Monocyte chemo attractant protein -1 (MCP-1), which triggers atherogenesis. The results of the current study correlates and suggests that smokers would have elevated MCP-1, which could predispose both periodontal disease as well as atherogenesis.

Previous study by Schenkein[19] reported that smoking influenced serum cell adhesion molecule levels and underscored the necessity of demonstrating that concentrations of these inflammatory markers, IgG, IgM, in periodontitis patients are independent of smoking. Heavy smokers in our present study had increased cell adhesion molecules, which could trigger inflammation in arteries.

A study by Doruk Erkan *et al*. reported that patients with APS are more prone to develop arterial and venous thrombi, and women with APS frequently experience recurrent spontaneous abortion.[20] Such patients usually have very high serum anticardiolipin levels.

George *et al*. showed that periodontal infections are associated with preterm labor, low birth weight, atherosclerosis, endothelial dysfunction, and myocardial infarction; although, cause and effect relationships are doubtful.[21]

The pathogenesis of APS remains unclear.[22] The autoantibodies reactive with phospholipids which target β2GPI are hypothesized to cause the disease by several pathways, including activation of endothelial cells, inducement of oxidant-mediated injury, or interference with the natural anticoagulant function of β2GPI.[23-25] This pathogenic mechanism elicited through these studies support that our subjects with tobacco users may initiate prothrombotic reactions and cyclic phenomenon.

Blank *et al*. and Schenkein *et al*. have proven that anticardiolipin can be stimulated by bacterial pathogens such as *Porphyromonas gingivalis*, *Hemophilus influenza* or

**Table 1: Anticardiolipin antibodies IgG, IgM levels in smokers and non-smokers with severe periodontitis**

| | Group | *n* | Mean | SD |
|---|---|---|---|---|
| IgG | Non-smokers | 20 | 25.0050 | 2.06588 |
| | Smokers | 20 | 15.8650 | 2.18951 |
| Mean CAL | Non-smokers | 20 | 2.8285 | 0.36212 |
| | Smokers | 20 | 2.8475 | 0.25194 |
| IgM | Non smokers | 20 | 23.4150 | 2.69332 |
| | Smokers | 20 | 14.8945 | 2.49259 |

*CAL=Clinical attachment loss; the results were statistically significant P<0.001 in smokers in severe periodontitis when compared to non-smokers*

**Table 2: Independent *t*-test in smokers and non-smokers participants**

| | *I*-test for equality of means | | | |
|---|---|---|---|---|
| | Sig (2-tailed) | Mean difference | Std. error difference | 95% confidence Lower |
| IgG equal variances assumed | 0.000 | 9.14000 | 0.67312 | 7.77734 |
| Equal variances not assumed | 0.000 | 9.14000 | 0.67312 | 7.77719 |
| IgM equal variances assumed | 0.000 | 8.52050 | 0.82058 | 6.85932 |
| Equal variances not assumed | 0.000 | 8.52050 | 0.82058 | 6.85900 |

*IgG levels were significantly increased (mean difference: 9.14000) compared to IgM levels (mean difference: 8.52050) in severe smokers*

*Neisseria gonorrheae*, and cytomegalovirus, which have peptide sequences similar to the TLRVYK peptide of β2GPI.[26] Three peptides sequence that are present in the arg-gingipain protease of the periodontal pathogen *Porphyromonas gingivalis* is similar to the TLRVYK peptide of β2GPI and can induce cross-reactive autoantibodies patients with periodontitis.[5,27]

Our previous study was to compare and correlate the levels of aCLAs in healthy, mild, moderate, and severe periodontitis patients. We found that patients with increased aCLAs have deeper pockets with more amount of attachment loss compared to healthy group. Severe periodontitis patients showed statistically significant elevated IgG and IgM aCLA ($P < 0.0001$) compared to other group as well as control groups.[28]

In 2008, Karnoutsos *et al*. conducted a study to determine the correlation between periodontal infection and increased concentration of anti-phosphorylcholine and aCLAs in serum patients with periodontitis. They found that patients with elevated anti-phosphorylcholine and aCLAs demonstrated increased pocket depth and greater mean attachment loss, compared to patients with normal levels of both antibodies. These results could be associated with an increased risk of stroke and atherosclerosis in patients with severe periodontitis.[11]

A study by Gustafson *et al*. evaluated the association between smoking and antiphosplipid antibodies in systemic lupus erythematosus and inferred that smoking was associated with the most pathogenic antiphospholipid antibodies.[14]

Another study done by Califano *et al*. to determine the reactivity of IgG concentration with recombinant *Porphyromonas gingivalis* hemagglutinin in 117 chronic periodontitis and 90 generalized aggressive periodontitis patients found that IgG exhibited reactivity with the organism in both chronic periodontitis and generalized aggressive periodontitis patients. However, they found that there were no significant relations.[29]

Based on these observations, smoking could raise aCLAs in severe periodontitis. In the present study, smokers with severe chronic periodontitis showed increase in anticardiolipin IgG IgM than that in non-smokers. Results infer that smokers are more prone to cardiovascular problems and systemic diseases. Exact cause for the increase in anticardiolipin in smokers with periodontitis is not known; hence, further research is necessary to establish the same. Limitations of this study were small sample size; hence, studies with large sample size with smokers are needed for better conclusive results.

## CONCLUSION

Results and statistical analysis showed an increase in aCLA in smokers with severe periodontitis. This indicates that these patients are more prone to coronary heart disease. This warrants further longitudinal studies with large sample size to investigate the relationship between coronary heart disease and smoking with severe periodontitis.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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