ORIGINAL ARTICLE

Evaluation of serum anticardiolipin antibody levels in patients with chronic periodontitis and essential hypertension: A clinico-biochemical study

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Abstract

Background: Periodontitis and hypertension, both of which are chronic inflammatory and asymptomatic in nature, have a high prevalence in the general population. Anticardiolipin (CL) antibodies are often directed against CL which is an important component of the inner mitochondrial membrane. Anti-CL antibodies can increase in chronic infection and inflammation, thrombosis, stroke, myocardial infarction, and atherosclerosis. Hence, the study was aimed to elucidate a link between periodontitis and systemic inflammatory status by estimating the serum anti-CL antibodies in patients with chronic periodontitis and essential hypertension.

Materials and Methods: A total of 60 patients who fulfilled the inclusion and exclusion criteria were selected and divided into four equal groups: (1) Healthy controls, (2) Periodontally healthy-hypertensive, (3) Chronic periodontitis-hypertensive, and (4) Chronic periodontitis-systemically healthy. Serum samples collected from the patients were analyzed for anti-CL antibodies using a specific enzyme-linked immunosorbent assay kit. Statistical method; the data obtained were subjected to statistical analysis using *t*-test.

Results: Anti-CL antibody levels were elevated in chronic periodontitis-hypertensive patients (Group 3) (3.7600-0.29665) compared with other groups, followed by (Group 2) periodontally healthy-hypertensive (2.5200-0.22804) ($P < 0.001$).

Conclusion: Chronic periodontitis results in increased systemic inflammatory burden that can further contribute to elevation of anti-CL antibodies in hypertensive patients.

Introduction

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation or recession or both.[1]

Hypertension is persistently raised blood pressure (BP) resulting from raised peripheral arteriolar resistance. In more than 90%, the cause of hypertension is unknown and it is termed essential hypertension which becomes more frequent as the age advances and appears to be related to genetics and obesity.[2]

It has been stated that the severity of periodontitis could be related to essential hypertension or *vice versa* but the molecular mechanism that links these diseases is not well characterized. Considering the inflammatory characteristics of essential hypertension and periodontitis, it is possible that the two diseases aggravate each other.[2]

Cardiolipin (CL) constitutes about 20% of the total lipid composition found in the inner mitochondrial membrane of all cells where it is essential for the optimal function of numerous enzymes that are involved in mitochondrial energy metabolism. Anti-CL antibodies are those often directed against CL, and elevated levels are often found in infections, systemic lupus erythematosus, anti-phospholipid syndrome, and Behcet's disease.[3]

It is hypothesized that the anti-CL antibodies may promote various pathogenic mechanisms through endothelial activation by upregulating adhesion molecule expression, monocytes, and platelet adherence and by accelerating atherosclerosis.[4]

The relationship between bacterial infections and anti-CL antibodies has been reported.[3] Systemic inflammation caused by chronic periodontitis might affect the serum anti-CL antibodies in individuals with essential hypertension, and elevated levels of this antibody might have a role in the association between chronic periodontitis and essential hypertension.[2]

Therefore, the aim of this study was to investigate the influence of chronic periodontitis on the levels of anti-CL antibodies in individuals with essential hypertension.

Materials and Methods

This was a cross-sectional study in patients visiting the Clinical Department of Periodontics between December 2008 and November 2009. The protocol was approved by the Institutional Human Ethical Committee of Rajah Muthiah Institute of Health Sciences.

Patient selection

The study population included 60 patients (32 males and 28 females) in the age 35-50 who were willing to participate in the study, and an informed consent was obtained from all participants. Patients were divided into four groups with 15 in each group as follows:

- Group 1: Periodontally and systemically healthy (6 males and 9 females)
- Group 2: Periodontally healthy-hypertensive (10 males and 5 females)
- Group 3: Chronic periodontitis-hypertensive (8 males and 7 females)
- Group 4: Chronic periodontitis-systemically healthy (8 males and 7 females).

Criteria for hypertension and chronic periodontitis

Hypertension

Those with systolic BP >140 mm Hg and/or a diastolic BP >90 mm Hg (as per the USA Joint National Committee guidelines) who were under antihypertensive medications for at least 2 years.[2]

Chronic periodontitis

Those with clinical signs of periodontitis including probing pocket depth (PPD) of ≥5 mm with bleeding on probing (BoP) and clinical attachment loss (CAL) of ≥3 mm at 30% of sites or more with radiographic evidence of bone loss.[2]

Exclusion criteria

1. Smokers
2. Individuals with secondary hypertension or other systemic diseases
3. Pregnant and lactating women
4. Those taking lipid lowering or immunosuppressive drugs
5. History of periodontal treatment in preceding 6 months and taking antibiotics or anti-inflammatory drugs within past 3 months of our study period.

Study design

In the initial visit, all patients who fulfilled the inclusion and exclusion criteria were examined clinically with a detailed medical history. According to the findings, they were assigned to one of the four groups mentioned. The clinical periodontal parameters, including PPD,[5] BoP,[6] and CAL[5] were determined at six sites per tooth and recorded in a pro forma. A series of full-mouth intraoral periapical radiographs was taken for those patients with periodontitis to ascertain bone loss and confirm the clinical diagnosis. All clinical examinations were performed by a single calibrated examiner (BP).

Sample collection

About 5 ml of blood sample was taken from each patient by venipuncture from an antecubital vein using Vacutainer tube.

Principle of enzyme-linked immunosorbent assay (ELISA)

All blood samples were analyzed in the same laboratory. An ELISA and standard serum were used to measure the IgG anti-CL levels using AESKULISA™ anti-CL IgG ELISA kit, and the levels of antibodies were expressed in G phospholipids units.

Serum samples diluted 1:101 were incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, would bind to the antigen. The unbound fraction was washed off, and then, antihuman immunoglobulins (conjugated) was incubated. This would react with the antigen-antibody complex of the samples in the micro plates if antibodies were present in the specimen. Unbound conjugate was washed off. Addition of tetramethylbenzidine substrate would generate an enzymatic calorimetric (blue) reaction, which would be stopped by diluted acid (color changes to yellow). The rate of color formation acts as a function of the amount of conjugate bound to antigen-antibody complex, and this was proportional to the initial concentration of the antibodies in patient's sample.

Statistical analysis

The data obtained from clinical and biochemical evaluations were subjected to statistical analysis, and *t*-test was applied to compare the values between the groups.

Results

When IgG anti-CL antibody values were compared between healthy controls (Group 1) (1.5200 ± 0.16432) and periodontally healthy-hypertensive group (Group 2) (2.5200 ± 0.22804), the anti-CL antibody levels were significantly higher in Group 2 [Table 1] ($P < 0.001$).

Table 2 shows a comparison of IgG anti-CL antibody levels between periodontally healthy-hypertensive (Group 2) and chronic

periodontitis-hypertensive (Group 3). The anti-CL antibody levels were significantly higher in Group 3 (3.7600 ± 0.29665) than Group 2 (2.5200 ± 0.22804) ($P < 0.001$).

Table 3 shows a comparison of IgG anti-CL antibody levels between healthy controls (Group 1) and chronic periodontitis-systemically healthy (Group 4). There was no significant difference between the Group 1 (1.5200 ± 0.16432) and Group 4 (1.4600 ± 0.11402) ($P = 0.305$).

Table 4 shows a comparison of IgG anti-CL antibody levels between chronic periodontitis-hypertensive (Group 3) and chronic periodontitis-systemically healthy (Group 4). The anti-CL antibody levels were significantly higher in Group 3 (3.7600 ± 0.29665) than Group 4 (1.4600 ± 0.11402) ($P < 0.001$).

Discussion

The present study was conducted to determine the anti-CL antibodies in serum of patients with hypertension, chronic periodontitis with and without hypertension, and compare the same with the healthy controls.

The study included 60 patients (32 males and 28 females) in the age 35-50 who were willing to participate in the study, and an informed consent was obtained from all participants. Patients were divided into four groups with 15 in each group as follows:

- Group 1: Periodontally and systemically healthy (6 males and 9 females)
- Group 2: Periodontally healthy-hypertensive (10 males and 5 females)
- Group 3: Chronic periodontitis-hypertensive (8 males and 7 females)
- Group 4: Chronic periodontitis-systemically healthy (8 males and 7 females).

Results of our study have shown that the serum anti-CL antibody levels were significantly higher in patients with chronic periodontitis and hypertension than those with only hypertension. These findings were similar to that of Türkoglu *et al.*,[2] who in their study observed elevated levels of serum anti-CL antibodies and oxidized low-density lipoprotein levels in chronic periodontitis patients with essential hypertension.

The elevation of anti-CL antibodies in sera of patients with chronic periodontitis and hypertension could be attributed to the reason for both periodontitis and hypertension are chronic in nature with acute episodic manifestations. Patients with periodontitis have not only local periodontal inflammation but also had higher inflammation in their blood stream. They also had higher concentrations of circulating granulocytes, CRP, fibrinogen, serum amyloid, and a host of other cytokines. Evidence is consistent with the notion that generalized severe periodontitis causes systemic inflammation and endothelial dysfunction.[7]

Furthermore, it was evident from the results of our study that the anti-CL antibody levels were significantly higher in periodontitis patients with and without hypertension as well as in patients with only hypertension when compared with periodontally and systemically healthy individuals. This was in accordance with the study done by Schenkein *et al.*,[3] who found elevated anti-CL antibodies in periodontitis group compared to healthy control.

Periodontal infection is associated with Gram-negative bacteremia, and this, in turn, leads to endothelial damage, platelet aggregation, and monocytic infiltration and proliferation. These monocytes produce various inflammatory cytokines such as interleukin-1, tumor necrosis factor-alpha, and interleukin-6, which can damage the endothelium. Damaged endothelium activates platelets which, in turn, results in platelet aggregation, and this, in turn, also potentiate thromboembolic events.[8] Similarly, Harvey *et al.*[9] studied anti-CL antibodies and serum adhesion molecule levels in patients with aggressive periodontitis and revealed that a subset of periodontitis patients with elevated anti-phospholipid antibodies could represent a subgroup with increased risk for obstetrical and cardiovascular sequel.

Chronic periodontitis might be associated with an increased level of anti-CL antibodies. This could be due to periodontal

Table 1: Comparison of anti-CL antibody levels (GPL units) between Groups 1 and 2

Groups	*n*	Mean	Standard deviation
Healthy control	15	1.5200	0.16432
Periodontally healthy-hypertensive	15	2.5200	0.22804

t-value 22.361, *P*<0.001 (S). CL: Cardiolipin, GPL: G phospholipids

Table 2: Comparison of anti-CL antibody levels (GPL units) between Groups 2 and 3

Groups	*n*	Mean	Standard deviation
2. Periodontally healthy-hypertensive	15	2.5200	0.22804
3. Chronic periodontitis-hypertensive	15	3.7600	0.29665

t-value 13.371, *P*<0.001 (S). CL: Cardiolipin, GPL: G phospholipids

Table 3: Comparison of anti-CL antibody levels (GPL units) between Groups 1 and 4

Groups	*n*	Mean	Standard deviation
1. Healthy control	15	1.5200	0.16432
4. Chronic periodontitis-systemically healthy	15	1.4600	0.11402

t-value 1.177, P=0.305 (NS). CL: Cardiolipin, GPL: G phospholipids

Table 4: Comparison of anti-CL antibody levels (GPL units) between Groups 3 and 4

Groups	*n*	Mean	Standard deviation
3. Chronic Periodontitis-hypertensive	15	3.7600	0.29665
4. Chronic Periodontitis-systemically healthy	15	1.4600	0.11402

t-value 20.172, *P*<0.001 (S). CL: Cardiolipin, GPL: G phospholipids

pathogens, especially Gram-negative bacteria, which induce the production of systemic inflammatory markers.[10]

Our results are consistent with those of Goteiner *et al.*,[11] who found increased levels of serum anti-CL antibodies in chronic periodontitis patients suffering from essential BP. They suggested that increasing anti-CL antibody levels could increase the risk of atherosclerosis in these patients.

The few limitations of our study were not taking genetic factors into consideration and not evaluated other systemic markers such as CRP levels as well as not doing a microbiological profile.

Conclusion

Within the few limitations of our study, it can be concluded that the results of our study clearly determined elevated anti-CL antibody levels in patients with chronic periodontitis and hypertension together followed by those with either hypertension or chronic periodontitis than the healthy controls. Thus, chronic periodontitis, when occurs in tandem with essential hypertension may considerably increase the risk of atherosclerosis due to increased anti-CL antibody levels. This gives rise to serious health concerns in patients with essential hypertension and chronic periodontitis.

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References

1. Newman MG, Takei HH, Carranza FA. Carranza's Textbook of Clinical Periodontology. 9th ed. Philadelphia, Pennsylvania: Saunders Publication; 2003. p. 67.
2. Türkoglu O, Baris N, Kütükçüler N, Senarslan O, Güneri S, Atilla G. Evaluation of serum anti-cardiolipin and oxidized low-density lipoprotein levels in chronic periodontitis patients with essential hypertension. J Periodontol 2008;79:332-40.
3. Schenkein HA, Berry CR, Burmeister JA, Brooks CN, Barbour SE, Best AM, *et al.* Anti-cardiolipin antibodies in sera from patients with periodontitis. J Dent Res 2003;82:919-22.
4. Mercanoglu F, Oflaz H, Ogun OZ, Gokbuget AY, Genchellac H, Sezer M, *et al*. Endothelial dysfunction in patients with chronic Periodontitis and its improvement after initial periodontal therapy. J Periodontol 2004;75:1694-700.
5. Armitage GC. The complete periodontal examination. Periodontol 2000 2004;34:22-33.
6. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975;25:229-35.
7. Tonetti MS. Periodontitis and risk for atherosclerosis: An update on intervention trials. J Clin Periodontol 2009;36 Suppl 10:15-9.
8. Montebugnoli L, Servidio D, Miaton RA, Prati C, Tricoci P, Melloni C, *et al*. Periodontal health Improves systemic inflammatory and haemostatic status in subjects with coronary heart disease. J Clin Periodontol 2005;32:188-92.
9. Schenkein HA, Best AM, Brooks CN, Burmeister JA, Arrowood JA, Kontos MC, *et al.* Anti-cardiolipin and increased serum adhesion molecule levels in patients with aggressive periodontitis. J Periodontol 2007;78:459-66.
10. Faghihi SM, Rokin AR, Ebrahimi R. Evaluation of serum anti-cardiolipin antibody titer in patients with chronic periodontitis. J Dent Univ Med Sci Tehran Iran 2009;6:2.
11. Goteiner D, Craig RG, Ashman R, Janal MN, Eskin B, Lehrman N. Endotoxin levels associated with high density lipoprotein, triglycerides and troponin in patients with acute coronary syndrome and angina; Possible contribution from periodontal sources. J Periodontol 2008;79:2331-9.

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