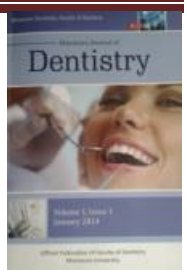

The effect of non-surgical periodontal therapy on serum anticardiolipin antibodies in chronic periodontitis patients associated with cardiovascular disease

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Abstract:

Background: Patients with periodontal diseases have high levels of serum anticardiolipin antibodies (aCLA). Anticardiolipin antibodies are class of antiphospholipid antibodies which cause thrombosis. Increased aCLA concentrations have a modulating role in cardiovascular diseases. The actual target antigen for these antibodies is β2-glycoprotein-I (β2GPI).Aim: of our study was to determine the effect of non surgical periodontal treatment (scaling and root planning) on serum aCLA level in chronic periodontitis patients associated with CAD. Subjects and methods: This study was conducted on forty individuals. Fifteen subjects suffering from chronic periodontitis and CAD were enrolled in the study as study group (group I), fifteen subjects suffering from chronic periodontitis as positive control group (group II) and ten healthy subjects as negative control group (group III). Clinical periodontal parameters (periodontal pocket depth, clinical attachment loss and bleeding on probing, plaque index) were recorded at baseline for all groups at baseline and six weeks after non surgical periodontal treatment for groupI and groupII. Serum samples were taken two times at baseline and six weeks after treatment for groupI and groupII and only once for group III. The patients were treated twice a week for three weeks. The samples were analyzed using Anticardiolipin ELISA kit. Results: All periodontal parameters (PI,BOP,PPD,CAL) and Laboratory analysis results (IgG, IgM, cholesterol, TGS, HDL, LDL) were reduced significantly by non surgical periodontal treatment. Conclusion: Scaling and root planning (SRP) is an effective treatment in chronic periodontitis patients associated with CAD. Successful periodontal therapy can improve the serum level of one of the inflammatory biomarkers (aCLA) which is involved in the cardiovascular problems.

Key words : chronic periodontitis, coronary artery disease, aCLA, β2-glycoprotein-I.

Introduction

Periodontitis is considered as a chronic inflammatory disease which is a common bone pathology in humans[1]. periodontal pathogens and their products cause local production of immune-inflammatory markers which in turn cause destruction of supporting tissues of the teeth[2]. The previous studies proved that there is association between periodontal disease and cardiovascular disease[3]. The link between cardiovascular disease and periodontitis is based on direct effect on endothelial cells via transient bacteremia or indirect action of inflammatory products on the endothelial cells[4].

The inflammatory cytokines, endotoxins, antigens which are produced by the periodontal pathogens lead to atherogenesis and thromboembolic events. Anticardiolipin antibodies (aCLA) could be responsible for these thrombotic events[5]. β2-glycoprotein-I-dependent anti aCLA which is a class of Antiphospholipid antibodies are found in 1%to 5% of systemically healthy individuals[6]. The levels of these antibodies can increase in several infectious diseases, systemic lupus erythrematosus or antiphospholipid syndrome[7]. Thrombosis, stroke, myocardial infarction, and atherosclerosis[22,23] are related to elevated levels of anti-CLA, which is similar to systemic conditions associated with periodontitis[8,9].

The natural anticoagulant that involves -dependent phospholipidβ (2GPI), present in the endothelial lining of blood vessels has an active role in the physiologic regulation of homeostasis caused by different stimuli and play a direct role in mediating platelet destruction[10]. pathogenic anti-CLA could be produced by a variety of microbial pathogens because these pathogens have similarities to the peptide sequences in b2GP1.TLRVYK is one such sequence in b2GP1 and also many microorganisms contain homologous peptide sequences to TLRVYK. This process is defined as molecular mimicry[11]. The target antigen for these autoantibodies is on the serum protein β2GP1, which binds to anionic lipids such as cardiolipin to form a complex that is recognized by these antibodies[12]. Wang et al. reported that patients with A.actinomycetemcomitans (A.a.) infection showed elevated antibody concentrations to the peptide SIRVYK, a sequence found in A.a. that is homologous to TLRVYK. Furthermore, anti-SIRVYK correlated with anti-TLRVYK in patients colonized with A.a. So, periodontal infection with A.a. contributes to the content of antibody reactive with β2GP1[13].

Subjects and Methods:

Patient selection: This study was carried on 40 patients. The patients were selected from those attending Oral Medicine and Periodontology Department, Faculty of Dentistry, Mansoura University as well as from the CVD Department of Faculty of medicine, Mansoura University. They were 18 males and 22 females. Their ages ranged from 35 to 55 years. The selected patients were divided into three groups.

Study groups: Group I (study group): This group includes fifteen patients with moderate chronic periodontitis and coronary heart disease. Coronary heart disease was included in the study according to the report of electrocardiogram (E.C.G), echocardiogram and angiogram and documented in the department of cardiology. **Group II (positive control group):** Fifteen patients were chosen as positive control group with chronic periodontitis without coronary heart disease. **Group III (negative control group):** Ten healthy subjects were chosen as negative control group with clinically healthy gingiva.

Inclusion criteria: (1)Age 35- 55 years. (2)Presence of at least 20 teeth, excluding third molars. (3)Cooperative patients.

Exclusion criteria: (1)Periodontal treatment or antibiotic therapy in the past 3 months before the study.(2)Patients who are pregnant or breast feeding.(3) Any other systemic diseases such as diabetes mellitus, rheumatoid artheritis, SLE and liver disease.(4) Mental disability

Clinical Assessment: Proper case history was taken from each patient, also the onset and duration of the patient's periodontal status was reported as well as any past dental treatment. Patients were exposed to thorough clinical oral and extraoral examination.

Periodontal Assessment: Periodontal indices: Plaque index [29], Bleeding on probing (BOP) [30], Probing pocket depth [31] and Clinical attachment level (CAL) [32].

Radiographic assessment: Panorama and periapical radiographs were taken to determine alveolar bone loss.

Periodontal treatment: Group I & II (study group and positive control group): patients were undergone non-surgical periodontal treatment (scaling &root planning) twice a week, oral hygiene measures, filling of carious teeth and correction of old restorations and blood samples were taken. Group III: blood samples were taken only once.

Blood sample collection: Five ml. of venous blood were collected from each patient at baseline examination and 6 weeks after non-surgical periodontal treatment using a 20-gauge needle with a 5-ml syringe, blood were obtained from the antecubital fossa by venipuncture method under standard aseptic precautions and then transferred to the plain evacuated tube. The tubes were immediately transferred to the laboratory and left to clot for 20 minutes at room temperature, centrifuged and clear sera are separated into two aliquots, one of them used for traditional laboratory investigation and the other kept frozen at -30°c till aCL assay.

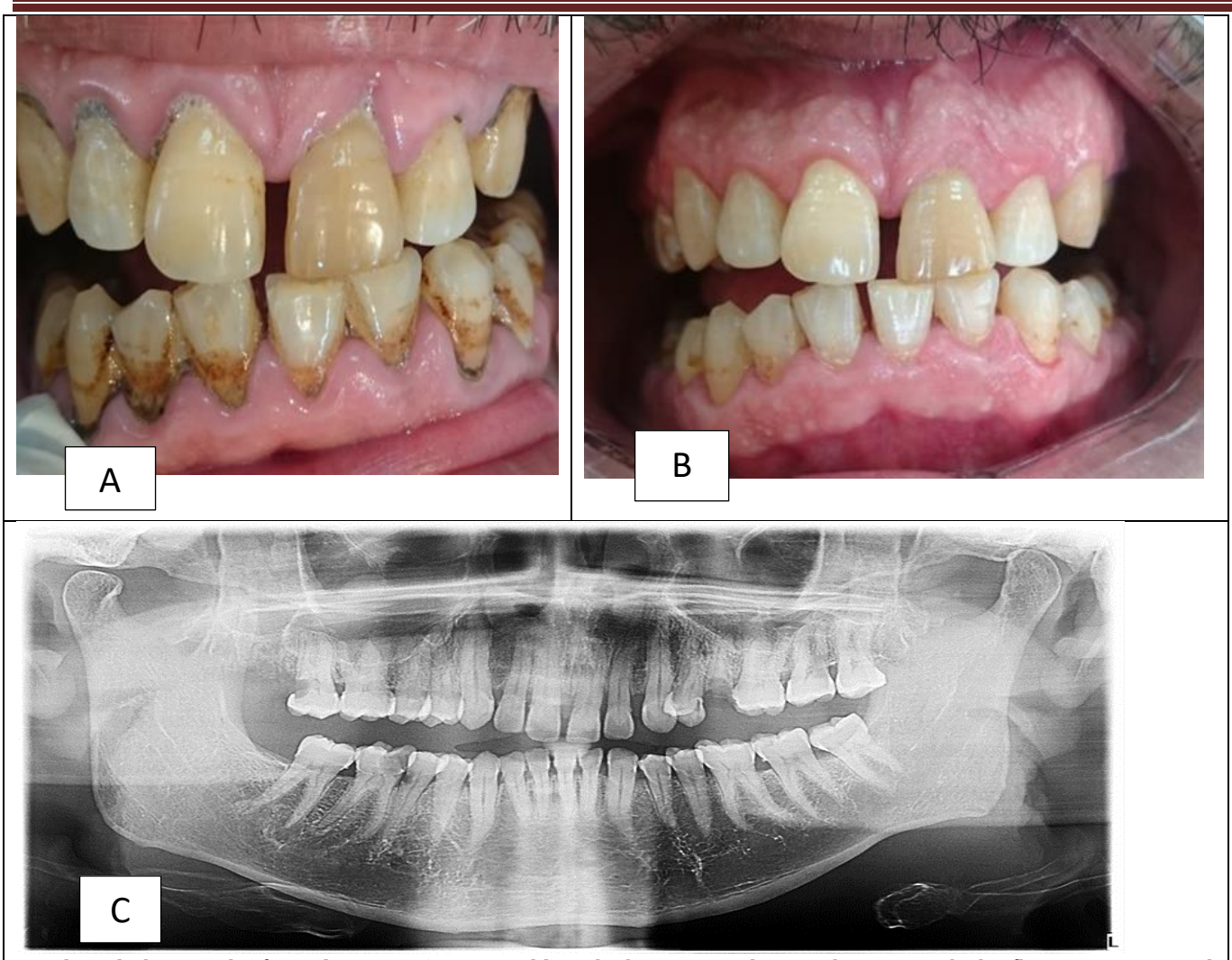
Laboratory assessment: Serum lipid profile analysis:

- Serum cholesterol, triglyceride (TG): were analyzed by enzymatic method.
- Serum high density lipoprotein(HDL-C): were analyzed by phosphotungistic acid and heavy metal precipitation of other lipoprotein then HDL-C will be assayed using enzymatic method.
- Low density lipoprotein (LDL-C): were calculated using the Friedwald equation: LDL-C=total cholesterol-(TG/5+HDL-C)

Anticardiolipin antibody analysis: Assay of Anti-Cardiolipin IgG/IgM (ORGENTEC Diagnostika GmbH) [14] : Serum samples were assayed for IgM and IgG aCL levels using a commercially available enzyme-linked immunosorbent assay (ELISA). The assays were conducted according to the manufacturer's instructions utilizing direct ELISA (sandwich) assay.

Statistical analysis: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0

Results: This study revealed that there was no statistically significant difference regarding IgG between groupI & groupII when measured at baseline. However IgM showed higher significant difference comparing groupI with group II (p=0.048) .The results of our study showed highly significant reduction in all clinical indices (PI,BOP,PPD,CAL) of studied groups when measured at six weeks after non-surgical periodontal treatment ($p \leq 0.001$). Also there was no statistically significant difference found in periodontal indices between groups. All values of laboratory analysis results (IgG, IgM, cholesterol, TGS, HDL, LDL) of studied groups measured at baseline were found to be improved significantly by non-surgical periodontal treatment as measured after 6 weeks. ($p \leq 0.001$).There was significant difference in laboratory analysis results (IgG, cholesterol, TGS, HDL, LDL) except IgM between groups. A statistically significant negative strong correlation was found between HDL&CAL among groupI ($r = -0.73$, $p = 0.025$*).Also a statistically significant negative strong correlation was found between HDL&CAL ($r = -0.93$, $p = <0.001$*). Moreover statistically significant positive strong correlation was found between Cholesterol & PI ($r = 0.68$, $p = 0.04$*). A statistically significant positive strong correlation was found between TG&PI among group II ($r = 0.71$, $p = 0.03$*).However, no statistically significant correlation was found between clinical indices and laboratory analysis results in group II 6 weeks after non-surgical periodontal treatment.



A: Clinical photograph of a male patient (45 years old) with chronic periodontitis showing marked inflammation, gingival recession and deposition of plaque and calculus. B: after treatment showing no signs of inflammation. C: panoramic radiograph

Discussion:

Epidemiological, pathological, microbiological and immunological studies demonstrated that infection by periodontopathic pathogens and their inflammatory markers which present in the blood have been associated with increased risk of CVD[15].Chronic exposure to Gram-negative bacteria, produces systemic inflammatory mediators[16]. The process of atherosclerosis is caused by either direct involvement of bacterial lipopolysaccharides and inflammatory cytokines or by indirect effect of antibacterial immune response[17]. Furthermore atheroma formation is directly and indirectly influenced by periodontal pathogens[16]. The present study focused on the assessment of circulating levels of aCLA in serum

among CP with CHD patients and CP patients and to evaluate the effectiveness of non-surgical periodontal treatment in adjusting the anticardiolipin concentration with the improvement in the periodontal parameters.

Regarding PI, BOP at baseline there was statistically significant difference when comparing group I with group II patients .This result was in agreement with those of **Pradeep et al;** who found that PI, GI, PBI in CP with CVD patients were highly significantly versus CP patients[18]. This finding was in contrast with **Zeynep TC et al;** they found no significant differences in PI, GI, BOP between chronic periodontitis with acute myocardial infarction patients and chronic

periodontitis patients [19].Regarding PPD & CAL at baseline no statistically significant differences were found between group I & group II at baseline, These results were in agreement with **Ashish et al;** they found no significant differences in PI, GI, PBI, PPD, CAL, in CP with CVD patients versus CP patients[20].

As expected when comparing group II with group III statistically significant differences were found in PI, BOP, PD and CAL at baseline. These results were in agreement

with **Wan Majdiah et al;** they found (PI, GI, PPD, and CAL) were significantly higher in CP group compared to non-CP group [21]. At baseline there were statistically significant differences in PI, BOP, PPD and CAL when comparing group I with healthy controls. This finding was in accordance with **Oya Tu¨rkoglu et al;** they found that the percentages of sites with BOP and supragingival plaque were significantly higher in the gingivitis-hypertension and periodontitis-hypertension groups than in the healthy controls and healthy-hypertension groups. Moreover the mean PPD&CAL scores were the highest in the periodontitis-hypertension group and significant differences were found in PD scores between the periodontitis-hypertension group and the other groups. Also they found that mean PPD scores in the healthy-hypertension and gingivitis-hypertension groups were significantly higher than in the healthy group[22]. The clinical parameters (PI, GI, BOP, PPD and CAL) in CP with CVD group, CP group and control group after 6 weeks of non-surgical periodontal treatment were found to be significantly decreased versus their levels at baseline. These results were in agreement with **Chaturvedi et al;** they found high significant improvement in PI, GI, PBI, and PPD, CAL, in CP with CVD patients after phase I periodontal therapy [23].

This could be due to scaling and root planning (SRP) is the the gold standard therapy for periodontitis. Removal of microbial plaque effectively by SRP leads to resolution of gingival inflammation. Moreover, improvement of the pocket depth due to the ability of the tissues to form thin junctional epithelium since the microbiologic-contaminated cementum layer was removed and the number of pathogenic microorganisms in the periodontal pocket was

reduced giving the tissue a chance to establish better repair[24]. At baseline IgM was found to be significantly higher in group I than in group II, however there was no significant difference found between groups in IgG , this is in accordance with **Tu¨rkoglu et al;** who found the mean serum IgM anti-CL level was significantly higher in the periodontitis-hypertension group compared to the other groups but there were no significant differences found in the mean serum IgG anti-CL and oxLDL levels among the study groups[22].

Significant differences were found between group II and group III regarding IgG & IgM at baseline These results were in agreement with **Wan Majdiah et al**; they found the level of IgG and IgM anti-CL antibodies were significantly higher in CP compared to non-CP group[21].

The possible explanation for the higher levels of serum anti-CL antibodies among chronic periodontitis patients versus healthy controls could be due to the fact that infectious disease including periodontitis may induce the production of these antibodies[25]. Blank et al. and Schenkein et al. had proven that anti-CL antibodies can be stimulated by bacterial pathogens such as Porphyromonas gingivalis, Hemophilus influenza or Neisseria gonorrhoeae, and cytomegalovirus, which have peptide sequences similar to the TLRVYK peptide of β2GPI [11].

Moreover significant differences were found between group I and group III regarding IgG &IgM antibodies , that was in agreement with **Sundaram R et al;** who found serum anti-CL antibody levels were significantly higher in patients with chronic periodontitis and hypertension than healthy subjects[26].

One mechanism by which aCLAs are associated with progression of atherothrombosis is their interaction with the endothelial cells. These cells have cell surface receptors called AnnexinII that can attract and bind to β2-glycoprotein I, which in turn can attract aCLAs[10]. This leads to endothelial cell activation, increased secretion of proinflammatory cytokines, release of tissue factor, and subsequent initiation of the coagulation cascade[10].These changes, along with endothelial dysfunction, eventually lead to a full-blown atheromatous lesion[27]. Damaged endothelium activates platelets which, in turn, results in platelet aggregation and this in turn also potentiate thromboembolic events[28].

In the present study non-surgical periodontal treatment resulted in significant reduction of levels of IgG & IgM aCLAs in groupI and groupII This was in accordance with **Gunupati et al;** who investigated the effect of periodontal therapy in patients suffering from acute myocardial infarction and chronic periodontitis. They showed significant alterations in the serum levels of IgG and IgM aCLA[29].

The effect of periodontal treatment on the improvement of endothelial dysfunction, the reduction of inflammatory biomarkers related to cardiovascular diseases (including C-reactive protein, interleukin-6), and carotid intima–media thickness. These studies showed positive results. Most of these interventional studies concluded that the body's inflammatory burden can be reduced by periodontal treatment[12,30–35]. So it can be postulated that the changes in gingival inflammation and plaque accumulation are partly attributed to the alterations in aCLA levels before and after treatment[30]. So, findings suggest severity of periodontitis should be considered when assessing the risk factors of coronary heart diseases, stoke and adverse pregnancy outcomes. Furthermore, recognition and treatment of periodontal diseases should become a part of routine therapy of those patients with the stated diseases[36].

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