

Effect of Intensive Non-Surgical Treatment on the Level of Serum Inflammatory Markers in Advanced Periodontitis

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Abstract:

Objective: To assess whether non-surgical periodontal treatment is associated with changes in serological markers of systemic inflammation.

Materials and Methods: Thirty-five systemically healthy subjects with severe generalized periodontitis meeting the inclusion criteria participated in a four-month single blind interventional trial of which thirty-two completed the study. Periodontal parameters and inflammatory markers [C-reactive protein (CRP) and plasma fibrinogen] and also the white blood cell count (WBC) were evaluated prior to and four months after delivery of intensive non-surgical periodontal therapy with simultaneous lavage of chlorhexidine 0.1% from the tip of the ultrasonic instrument into the pockets.

Results: Significant differences in serum CRP levels were observed four months after treatment compared to the baseline (1.85, SD=1.93 vs 2.46, SD=2.32, respectively, $P<0.0001$). Periodontal treatment also resulted in a significant difference in WBC and neutrophil counts compared to the baseline ($P<0.0001$). The reduction in fibrinogen levels was not significant at the end of the research period. Significant improvement in the pocket probing depth and clinical attachment level for pockets with initially 4-6 mm and then more than 7 mm depth was observed. Changes in plaque and bleeding scores were also statistically significant (82.75 vs. 35.84 and 19.03 vs. 1.81, respectively).

Conclusion: Periodontal treatment is effective in reducing CRP levels and white blood cell count, while fibrinogen levels are not influenced by periodontal therapy. Periodontal treatment may therefore decrease the systemic inflammatory burden in patients with advanced periodontitis.

Key Words: Periodontal Index; Periodontal Diseases; C-Reactive Protein; Root Planing

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INTRODUCTION

Emerging evidence from epidemiologic studies indicates that periodontal infection is associated with a moderate systemic inflammatory response [1-3]. In more severe forms of this disease the ulcerated epithelial lining of the periodontal pocket may constitute a substantial surface area through, which lipopoly-

saccharide and other bacteria-derived antigenic structures challenge the immune system, triggering the synthesis of several pro-inflammatory cytokines among which interleukin-6 (IL-6) named as messenger cytokine can travel from local inflamed tissue to the liver where it initiates a change in the program of protein synthesis from the housekeeping pro-

tein, albumin, to proteins characteristic of the acute phase response, including (but not limited to) C-reactive protein (CRP), fibrinogen and haptoglobin.

Made of five 23-KDa subunits, CRP, a member of pentraxin family of proteins, is a globulin which signals the inflammatory state in the body, and is now considered as a major cardiovascular risk factor [4,5]. Like other acute phase proteins, liver is the primary source of CRP synthesis; however, recent data indicate that it can be derived from the cells of human atherosclerotic intima [6]. The role of CRP in the pathogenesis of atherosclerosis via different mechanisms, such as augmenting the expression of local adhesion molecules, and LDL uptake by macrophages as well as its interaction with complement at the vascular level has documented this reactant protein not simply as a marker of inflammation, but also as a predictor of future cardiovascular events[7,8].

Based on a large series of prospective epidemiological studies, CRP, when measured with new high-sensitivity assays (hs-CRP), strongly and independently predicts risk of myocardial infarction, stroke, peripheral arterial disease and sudden cardiac death even among apparently healthy individuals [9,10]. In a previous study, we found that among acute myocardial infarction patients matched for traditional

CVD risk factors, case with having periodontal disease, had significantly higher levels of CRP than those without periodontal disease. The patients were also matched for the infarct size [11].

The primary data on the association between periodontal disease and elevated serum CRP levels was reported by Ebersole et al [1], which suggested that certain treatments are capable of lowering serum CRP levels. In their pilot study, this inflammatory marker was reduced in the serum of patients after non-surgical periodontal treatment along with non-steroidal anti-inflammatory drug (flurbiprofen) intake. Since then numerous studies have been conducted investigating the impact of periodontal infection and the total inflammatory burden on the host and whether the interventional strategies aiming at eradication of bacterial niches in the periodontal pockets reverse the resultant systemic inflammatory state [12-14]. However, based on a recent meta-analysis conducted in Medline (1960 to 2005), results from randomized controlled trials and single cohort studies do not show any significant difference in serum CRP levels before and after periodontal treatment [15]. Mentioning these controversial results, the aim of this prospective intervention trial was to assess whether intensive non-surgical periodontal treatment

Table 1. Values of inflammatory markers and periodontal parameters at baseline and 4-months after periodontal therapy.

Parameters	Baseline		4 th Month		P Value
	Mean	SD	Mean	SD	
CRP (mg/l)	2.46	2.31	1.85	1.93	<0.0001
Fibrinogen(mg/dl)	330.87	58.43	329.68	68.95	0.929 ^{NS}
WBC	7512.5	1769.4	6487.5	1261.27	<0.0001
Neutrophil	4553.06	1658.39	3571.09	764.30	<0.0001
PI	82.75	12.91	35.84	14.73	<0.0001
BOP	19.03	2.13	1.81	1.76	<0.0001
PPD (% 4-6 mm)	39.44	14.32	19.64	13.72	<0.0001
PPD (% ≥7 mm)	10.47	9.33	2.07	3.81	<0.0001
CAL (% 4-6 mm)	48.58	12.98	22.57	9.87	<0.0001
CAL (% ≥7 mm)	13.11	10.94	2.93	4.42	<0.0001

SD=Standard Deviation, PI=Plaque Index, BOP=Bleeding On Probing, PPD=Pocket Probing Depth, CAL=Clinical Attachment Level, NS= Not statistically Significant

was associated with changes in serological markers of systemic inflammation, i.e., CRP, fibrinogen and white blood cell count in otherwise healthy individuals.

MATERIALS AND METHODS

A prospective, longitudinal, single blind, interventional trial with a four-month follow up was conducted among thirty-five systemically healthy subjects (18 men, 17 women) in the age range of 19-56 years. The participants were recruited from non-smoking subjects of severe (probing pocket depth and attachment loss more than 5 mm, and radiographic evidence of alveolar bone loss), generalized (more than 30% of sites affected), periodontitis with at least 20 vital teeth referred to the Department of Periodontology, Guilan University of Medical Sciences (GUMS), School of Dentistry. All the subjects had BMI (body mass index) of 20 to 27 kg/m².

Exclusion criteria were considered as: (i) any periodontal treatment in the preceding six months (ii) known systemic diseases or any chronic inflammatory/infectious conditions such as arthritis, bronchitis, mucocutaneous conditions and sinusitis (iii) pregnancy or lactation (iv) any antimicrobial treatment in the previous six months or treatment with any medication affecting the serum level of inflammatory makers such as anti-inflammatories, hormone replacement and steroids, statins, immunosuppressants and anti-coagulants. All patients gave written informed consent. This study was reviewed and approved by the GUMS ethics committee.

Periodontal assessment including the pocket probing depth (PPD), clinical attachment level (CAL) – recorded at six sites per tooth; namely, mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual and CAL measured from the cemento enamel junction (CEJ) – bleeding on probing (Lenox & Kopczyk) for six anterior teeth of both jaws was carried out by a single examiner blinded to the study protocol at baseline and four months following periodontal treatment using a manual Williams periodontal probe (Hu-Friedy, IKK UNITY, USA) with a probe tip of 0.4 mm in diameter and a rounded working end. Plaque index (O’Leary) was recorded for all scorable, fully erupted teeth. The American Heart Association (AHA) and the Center for Disease Control (CDC) in a joint consensus report, released a guideline for cardiovascular risk assessment, which has identified three different risk categories merely based on serum CRP levels. Based on CRP levels; lower than 1 mg/l, 1-3 mg/l and higher than 3 mg/l, patients are categorized as low risk, medium risk and high risk for future cardiovascular disease and/or events, respectively [16]. We further categorized the patients according to this guideline, before and after intervention.

Each subject provided a 5 ml venous blood sample at baseline and underwent a non-surgical periodontal treatment starting with intensive oral hygiene instruction followed by deep ultrasonic instrumentation using a magneto-restrictive device equipped with constant flow of 0.1% chlorhexidine digluconate from its tip into the pockets during instrumentation.

Table 2. Mean decrease in PPD and attachment level gain for 4-6 mm and more than 7 mm pocket depths following treatment.

Parameters	Min	Max	Mean	SD
PPD Decrease (4-6 mm)	1.1	4.2	2.37	0.79
PPD Decrease (≥ 7 mm)	1.1	5.3	2.82	0.9
CAL Gain (4-6 mm)	1.2	3.9	2.38	0.74
CAL Gain (≥ 7 mm)	1	4.7	2.85	0.87

SD=Standard Deviation, PPD=Pocket Probing Depth, CAL=Clinical Attachment Level

Mechanical therapy was completed in two unlimited time appointments with one-week interval between the sessions. Subjects were instructed to brush their teeth and tongue with a soft toothbrush and Listerine toothpaste. An Oral-B anti-septic mouthwash (Oral-B Laboratories, Ireland) was also prescribed for the patients to use for two weeks. Plaque control and oral hygiene instructions were repeated appropriately during the four-month follow up, after which a full periodontal reassessment was carried out and further blood samples were taken. Peripheral blood samples were taken and frozen at -20°C until use. CRP determination was performed with a highly sensitive enzyme-linked immunosorbent assay (ADBC, Diagnostics Biochem. Canada Inc., detection limit of 10 ng/l) and the plasma fibrinogen level was performed using the digital coagulation technique (Plasma Te clot, Manufactured by TECO GmbH, Germany). WBC and neutrophil counts were detected immediately, using a cell counter device (KX21N, Sysmex, Kobe, Japan) and cell differentiation was controlled with a peripheral blood smear.

Using previous estimates of variance for the primary outcome such as changes in the level of inflammatory markers following periodontal treatment [17], the sample size was based on formal calculation methods. Baseline and reassessment data were compared using paired t-test. Data were analyzed by SPSS version 16 and a p value of 0.05 was assumed significant.

RESULTS

Totally, 32 participants completed the study. Three patients refused to give the second blood sample and were excluded from the study population. Together with positive changes in parameters of periodontal disease, non-surgical periodontal therapy resulted in a significant reduction in the circulating levels of CRP and WBC/neutrophil counts. No significant changes occurred in the concentration of fibrinogen four months after the completion

of periodontal treatment (Table 1).

Table 1 also illustrates the findings of clinical periodontal parameters at baseline and at the reassessment visit, indicating significant improvements in plaque, bleeding scores and also in the percent of pockets with the initial PD of 4-6 mm and more than 7 mm ($P < 0.0001$).

There were similar findings for CAL gain in the sites initially having an attachment loss of 4-6 mm and more than 7 mm. Table 2 illustrates mean reduction values for PPD and mean CAL gain in pockets with an initial PD and CAL of more than 4 mm.

According to AHA/CDC categorization, at baseline, 12 patients were in the low risk group, and 10 in each of the medium and high risk groups. Four months after intervention, there was a shift from medium and high toward the lower risk groups.

DISCUSSION

This study aimed at examining the effect of extensive full mouth non-surgical periodontal treatment on the level of systemic inflammatory indicators, which also play an important role as risk markers for cardiovascular disease. We showed that eradication or suppression of periodontal pathogens not only from the periodontal pockets but also from all their intra-oral habitats (mucous membranes, tongue and saliva) by applying full mouth disinfection in a relatively short time period [18], was associated with a significant decrease in serum CRP levels and WBC/neutrophil counts in otherwise healthy individuals affected by severe generalized periodontitis.

Accumulating evidence has shown that treatment of severe periodontitis is associated with subsequent changes in the level of serum inflammatory markers [12,14,19-22]. However, some other investigations have reported controversial results [23,24]. Ide et al [24] reported no statistically significant changes in the level of CRP, IL-6 and IL-1 β following a single course of periodontal treatment. Al-

though they excluded current smokers, they did not assess the effect of obesity or hypertension. Obesity is one of the typical subclinical conditions associated with higher CRP values. In our study, all the subjects had a BMI between 20 and 27 kg/m²; therefore, they were not apparently obese.

Offenbacher et al [25], in a secondary cardiac event prevention model, showed that periodontal intervention significantly lowers the hs-CRP levels in patients with baseline CRP values higher than 3 mg/l, after 6 months. However, as a strong confounder, obesity nullified the periodontal treatment effects on hs-CRP reduction in their study.

Furthermore, we also excluded subjects with any other infectious or inflammatory conditions or those taking any medications, which could affect the level of systemic variables under investigation and subjects with hypertension.

In a recent case-control study, Vidal et al [26] measured the serum level of CRP, IL-6 and fibrinogen after non-surgical periodontal therapy in refractory arterial hypertensive patients affected by severe periodontitis. They reported significant decrease in plasma variables 3 months after intervention in addition to the improvement of periodontal clinical results they had reached.

On the other hand, according to Jastrzebski et al [27], in the presence of hypertension and other cardiovascular risk factors such as hypercholesterolemia, obesity, and diabetes mellitus, the impact of periodontal treatment on the total inflammatory burden (as was implicated by serum CRP and fibrinogen levels) is small.

Unlike Jastrzebski et al [27], Lalla et al [28], in a pilot study showed decreased secretion of serum CRP and soluble E selectin, by blood-derived macrophages in diabetic patients four weeks after full mouth subgingival debridement.

The present study may be compared with that

of Marcaccini et al [21] with respect to CRP, Ellis et al [29], and also Buhlin et al [30] with respect to both CRP and fibrinogen levels and WBC counts, following periodontal treatment. Similar to our findings, in the two studies mentioned above, no significant changes were induced by periodontal therapy on fibrinogen levels.

Despite the consisting data indicating the predictive value of plasma fibrinogen levels in CVD/stroke risk assessment, and also the cumulative effect of hs-CRP and fibrinogen in risk prediction, fibrinogen evaluation has found limited use in clinical practice because of suboptimal assay standardization; therefore consistency across reference laboratories remains poor [22]. Since unlike CRP, highly sensitive assays for this marker have not been developed, it seems possible that with large-scale studies, significant changes in fibrinogen levels also become evident. In this regard, it should also be mentioned that application of non-surgical periodontal treatment leaves behind the chance of some residual diseased sites, especially in initially deep pockets, and this may have influenced our results.

One of the other findings of the present study, although not measured as a primary outcome, was the change occurring in the CRP-associated CVD risk among patients following successful non-surgical periodontal intervention, which is in agreement with that of D'Aiuto F et al [14].

CONCLUSION

Within the limitations of this study, successful non-surgical periodontal treatment results in significant decrease in serum CRP levels and WBC/neutrophil counts in otherwise healthy subjects.

Due to the relatively small sample size, the results obtained here need to be interpreted with caution. In addition, it should be emphasized that in order to indefinitely demonstrate the potential benefits of periodontal therapy to

reduce the level of serum inflammatory markers, and also of greater concern, whether treatment improves the overall cardiovascular risk, large multi-center randomized trials are suggested.

Given the high prevalence of periodontal disease, and in the light of predictable periodontal treatment posing negligible risk and leading to a high standard of oral health, we suggest periodontal treatment to be considered as a part of the cardiovascular preventive program, both in the future study designs and clinical practice.

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REFERENCES

1-Ebersole JL, Machen RL, Steffen MJ, Willmann DE. Systemic acute-phase reactants, C-reactive protein and haptoglobin in adult periodontitis. *Clin Exp Immunol* 1977 Feb;107(2):347-52.

2-Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, van der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *J Periodontol* 2000 Oct;71(10):1528-34.

3-Slade GD, Offenbacher S, Beck JD, Heiss G, Pankow JS. Acute-phase inflammatory response to periodontal disease in the US population. *J Dent Res* 2000 Jan;79(1):49-57.

4-Ridker PM, Libby P. Risk factors for atherosclerotic disease. In: Zipes DP, Libby P, Bonow

RO, Braunwald E, editors. A textbook of cardiovascular medicine. 7th ed. Philadelphia: Elsevier Saunders; 2005. p. 939-58.

5-Lindhe J, Lang NP, Karring T. Clinical periodontology and implant dentistry. 5th ed. Oxford: Blackwell Munksgaard; 2008. p. 149-56.

6-Calabro P, Willerson JT, Yeh ET. Inflammatory cytokines stimulated CRP production by human coronary artery smooth muscle cells. *Circulation* 2003 Oct;108(16):1930-2.

7-Zwaka TP, Hombach V, Torzewski J. C-reactive-mediated LDL uptake by macrophages: implications for atherosclerosis. *Circulation* 2000 Mar 6;103(9):1194-7.

8-Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 2000 Oct 31;102(18):2165-8.

9-Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 2002 Nov 14;347(20):1557-65.

10-Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocystein, lipoprotein a, and standard cholesterol screening as predictors of peripheral arterial disease. *JAMA* 2001 May 16;285(19):2481-5.

11-Radafshar G, Shad B, Mirfeizi M. Association between periodontal disease and elevated C-reactive protein in acute myocardial infarction patients. *J Dent Tehran Uni Med Sci* 2006;3(3):129-35.

12-Tuter G, Kurtis B, Sedar M, Aykan T, Okyay K, Yucel A, et al. Effects of scaling and root planing and sub-antimicrobial dose doxycycline on oral and systemic biomarkers of disease in patients with both chronic periodontitis and coronary artery disease. *J Clin Periodontol* 2007 Aug;34(8):673-81.

13-Yamazaki K, Honda T, Oda T, Ueki-Maruyama K, Nakajima T, Yoshie H, et al. Effect of periodontal treatment on the C-reactive protein and proinflammatory cytokine levels in Japanese periodontitis patients. *J Periodontol* 2005 Feb;

- 40(1):53-8.
- 14-D'Aiuto F, Ready D, Tonetti MS. Periodontal disease and C-reactive protein-associated cardiovascular risk. *J Periodontol Res* 2004 Aug;39(8):236-41.
- 15-Ioannidou E, Malekzadeh T, Dongari-Bagtzoglou A. Effect of periodontal treatment on serum C-reactive protein levels: a systemic review and meta-analysis. *J Periodontol* 2006 Oct;77(10):1635-42.
- 16-Shah SH, Newby LK. C-reactive protein: a novel marker of cardiovascular risk. *Cardiol Rev* 2003 Jul-Aug;11(4):169-79.
- 17-Iwamoto Y, Nishimura F, Soga Y, Takeuchi K, Kurihara M, Takashiba S, et al. Antimicrobial periodontal treatment decreases serum C-reactive protein, tumor necrosis factor alpha, but not adiponectin in patients with chronic periodontitis. *J Periodontol* 2003 Aug;74(8):1231-6.
- 18-Quirynen M, Mongardini C, de Soete M, Pauwels M, Couke W, van Eldere, et al. The role of chlorhexidine in the one-stage full-mouth disinfection treatment of patients with advanced adult periodontitis. Long-term clinical and microbiological observations. *J Clin Periodontol* 2000 Aug;27(8):578-89.
- 19-D'Aiuto F, Parkar M, Andreou G, Brett PM, Ready D, Tonetti MS. Periodontitis and systemic inflammation: control of the local infection is associated with a reduction in serum inflammatory markers. *J Dent Res* 2004 Feb;83(2):156-60.
- 20-Mattila K, Vesanen M, Valtonen V, Nieminen M, Palosuo T, Rasi V, et al. Effect of treating periodontitis on C-reactive protein levels: a pilot study. *BMC Infect Dis* 2002 Dec 10;2:30.
- 21-Marcaccini AM, Meschiari CA, Sorgi CA, Saraiva MC, de Souza AM, Faccioli LH, et al. Circulating interleukin-6 and high-sensitivity C-reactive protein decrease after periodontal therapy in otherwise healthy subjects. *J Periodontol* 2009 Apr;80(4):594-602.
- 22-Radvar M, Tavakkol-Afshari J, Bajestan MN, Naseh MR, Arab HR. The effect of periodontal treatment on IL-6 production of peripheral blood monocytes in aggressive periodontitis and chronic periodontitis patients. *Iran J Immunol* 2008 Jun;5(2):100-6.
- 23-Behle JH, Sedaghatfar MH, Demmer RT, Wolf DL, Celenti R, Kebschull M, et al. Heterogeneity of systemic inflammatory responses to periodontal therapy. *J Clin Periodontol* 2009 Apr;36(4):287-94.
- 24-Ide M, McPartlin D, Coward PY, Crook M, Lumb P, Wilson RF. Effect of treatment of chronic periodontitis on levels of serum markers of acute phase inflammatory and vascular responses. *J Clin Periodontol* 2003 Apr;30(4):334-40.
- 25-Offenbacher S, Beck JD, Moss K, Mendoza L, Paquette DW, Barrow DA, et al. Results from the periodontitis and vascular events (PAVE) study: a pilot multicentered, randomized, controlled trial to study effects of periodontal therapy in a secondary preventive model of cardiovascular disease. *J Periodontol* 2009 Feb;80(2):190-201.
- 26-Vidal F, Figueredo CM, Cordovil I, Fischer RG. Periodontal therapy reduces plasma levels of interleukin-6, C-reactive protein and fibrinogen in patients with severe periodontitis and refractory arterial hypertension. *J Periodontol* 2009 May;80(5):786-91.
- 27-Jastrzebski M, Zaleska M, Klocek M, Stolarz K, Wojciechowska W, Olszanecka A, et al. Should dental treatment be considered for lowering inflammatory markers in hypertensive patients? *Int J Cardiol* 2009 Mar 6;132(3):439-41.
- 28-Lalla E, Kaplan S, Yang J, Roth GA, Papapanou PN, Greenberg S. Effects of periodontal therapy on serum C-reactive protein, sE-selectin, and tumor necrosis factor-alpha secretion by peripheral blood-derived macrophages in diabetes: a pilot study. *J periodontal Res* 2007 Jun;42(3):274-82.
- 29-Ellis J, Averley P, Preshaw PM, Steele JG, Seymour RA, Thomason JM. Change in cardiovascular risk status after dental clearance. *Br Dent J* 2007 May 12;202(9):543-4.
- 30- Buhlin K, Hultin M, Norderyd O, Persson L, Pockley AG, Pussinen PJ, et al. Periodontal treatment influences risk markers for atherosclerosis in patients with severe periodontitis. *Atherosclerosis* 2009 Oct;206(2):518-22.