

Statistical Study on Serum CRP levels in Aggressive & Chronic Periodontitis: Pre & Post Non-Surgical Periodontal Therapy

Varshal J Barot* and Sarath Chandran

Department of Periodontics & Implantology, Manubhai Patel Dental College & Hospital, Vadodara, Gujarat, India

*Corresponding author: Varshal J Barot, MDS, Department of Periodontics & Implantology, Manubhai Patel Dental College & Hospital, Vadodara, Gujarat, India, Tel: +91 9558805347; E-mail: varshalb@gmail.com

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Abstract

Background: Elevated serum C-reactive protein (CRP) is regarded as a risk predictor for cardiovascular diseases. A positive association between circulating CRP levels and periodontal disease may be responsible. The aim of this study was to determine the effect of non-surgical periodontal therapy on serum CRP, WBC and OHI-S level in patients with aggressive and chronic periodontitis.

Methods: Total 57 subjects, divided into 3 groups based on the diagnosis into: Group A- aggressive periodontitis (n=17), Group B- chronic periodontitis (n=20) and Group C- non-periodontitis (n=20). After clinical data were recorded, venous blood was taken at baseline and 1 month after nonsurgical periodontal therapy. Periodontal examination included probing depth at baseline and OHI-S level was recorded at baseline and after 1 month. Blood was analysed to determine serum CRP and white blood cells (WBC) count.

Results: Mean WBC level: In group A, at baseline is $1.03E4 \pm 1850.85$ whereas it is 7976.47 ± 1538.47 after one month and in group B, at baseline is 9464.00 ± 1904.62 whereas it is 8510.00 ± 1778.79 after one month.

Mean OHI-S level: In group A, at baseline was 2.8847 ± 0.83 whereas it was 0.7029 ± 0.30 after one month and in group B, at baseline was 3.1480 ± 0.87 whereas it was 0.6465 ± 0.40 after one month. Treatment resulted in reduction of WBC count and oral hygiene index- simplified (OHI-S) score levels ($p < 0.05$).

Mean CRP level: In group A, at baseline was 5.091 ± 10.02 whereas it was 3.0153 ± 3.37 after one month and in group B, at baseline was 2.658 ± 1.82 whereas it was 3.0030 ± 2.24 after one month. In group C at baseline was 1.917 ± 0.79 . However, there were statistically no significant changes in levels of serum CRP in aggressive and chronic periodontitis ($p > 0.05$).

Conclusion: Both forms of periodontitis are associated with increased systemic inflammatory response with aggressiveness of disease progression, but improvement in periodontal health did not influence the levels of serum CRP.

Keywords: Acute-phase proteins; C-reactive protein; non-surgical periodontal therapy; periodontal disease

Introduction

Periodontitis is the most common, destructive, inflammatory diseases of supporting tissues of the teeth in humans, with profound effects on general health [1]. The role of micro-organisms in the initiation of periodontitis is well documented. They induce tissue destruction indirectly by activating host defense cells, which in turn produce and release mediators of tissue destruction. In general, IL-6 like cytokines synergizes with IL-1 like cytokines to induce type I acute-phase proteins/reactants. These cytokines may enter the blood stream as shown by the high levels of inflammatory biomarkers in both gingival tissues and serum. The acute-phase reactants receiving the most attention are C-reactive protein (CRP), plasminogen-activator 1 (PAI-1), and fibrinogen [2]. Moreover, studies have shown that levels of serum CRP in patients with periodontal diseases are elevated and constitute a risk predictor for cardiovascular diseases (CVD). The exact

role of periodontal diseases in the progression of CVD, if existing, is still poorly understood [3].

Moderately elevated numbers of leukocytes have been associated with an increased risk for cardiovascular diseases. Also, since higher number of leukocytes increases the blood rheology; more cells make the blood more viscous, and cells may adhere to endothelial cells lining the blood vessels, thereby decreasing the blood flow which can lead to atherosclerotic plaque formation [4].

Periodontal diseases being a plaque induced infection, the basic approach to them has always been and remains the removal of supra and sub-gingival bacterial deposits by scaling and root planning. Nonsurgical mechanical periodontal treatment is the cornerstone of periodontal therapy and the first recommended approach to the control of periodontal infections. Nonsurgical periodontal therapy has evolved over the years, and it is still considered to be the gold standard to which other treatment modalities are compared [5].

Most studies till date have included patients with chronic periodontitis and few investigators have studied CRP levels in subjects

with aggressive periodontitis. However, comparison of CRP in these two forms of periodontitis with effect of non-surgical periodontal therapy on systemic levels of CRP has been rare.

Thus, the aim of the present study is to determine the effect of non-surgical periodontal therapy on serum CRP level in patients with aggressive and chronic periodontitis.

Materials and Methods

An interventional study was designed and conducted in the Department of Periodontics & Implantology, Manubhai Patel Dental College & Hospital, Vadodara. Total 57 subjects (20 in each group) with 3 dropouts in group A were included and divided into 3 groups (Figure 1).

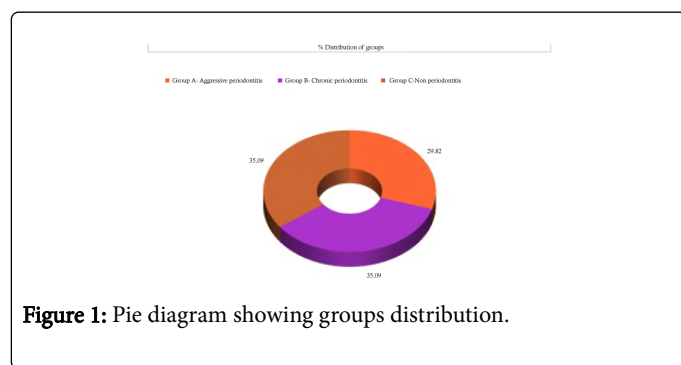


Figure 1: Pie diagram showing groups distribution.

Group A- Aggressive periodontitis (17)

Group B- Chronic periodontitis (20)

Group C- Non-periodontitis (20)

Verbal and written informed consent was obtained from all subjects prior to their enrollment in the study. The study was reviewed by the board of ethical committee for the dental college and was envisaged after obtaining the clearance. Subjects were selected from the outpatient department (OPD) after fulfilling the inclusion & exclusion criteria for the period of one year. Participation of the subjects in this study did not affect any treatment decisions regarding medical care.

Inclusion criteria

1. Systemically healthy subjects \geq 18 years of age.
2. At least 20 natural teeth present in oral cavity.
3. Subjects diagnosed clinically and radiographically with aggressive and chronic periodontitis. (According to American Academy of Periodontology 1999 classification)
4. Aggressive periodontitis: Subjects with at least 8 teeth with probing depth \geq 6mm and radiographic evidence of alveolar bone loss in at least three teeth other than first molars or incisors.
5. Chronic periodontitis: Subjects with at least four teeth with a pocket depth of \geq 4 mm and loss of attachment of \geq 3 mm in three sites of each involved tooth.
6. Non-periodontitis: Subjects with healthy/ mild gingivitis condition of periodontium.

Exclusion criteria

1. History of systemic diseases that may affect CRP (eg: impaired glucose tolerance, diabetes mellitus or endocrine diseases, nephrotic syndrome, chronic renal disease and cardiovascular disease).
2. Recent history or presence of acute or chronic infection.
3. Systemic antibiotic treatment or any other anti-inflammatory medication within the previous 3 months.
4. History of any periodontal therapy within 6 months.
5. Smoking within previous 3 months.
6. Pregnant or lactating women.
7. Bleeding disorders.

Armamentarium used in the study (Figure 2).



Figure 2: Armamentarium used in the study.

1. Gloves and mask
2. Mouth mirror
3. UNC-15 periodontal probe
4. Kidney tray
5. Alcohol soaked cotton swabs
6. Tourniquet
7. Disposable syringe and needle
8. Plain bulbs
9. Sterilium
10. CRP kit

Procedure

1. Total 57 subjects were divided into 3 groups based on the diagnosis into aggressive periodontitis, chronic periodontitis and non-periodontitis.
2. Non-surgical periodontal therapy includes oral hygiene instructions and supra & sub-gingival scaling and root planing under local anaesthesia in 1 week of first appointment.
3. Blood was collected from subjects by a single venepuncture in the antecubital fossa in plain vials, at baseline and 1 month after non-surgical periodontal therapy.

Clinical parameters

The periodontal examination included clinical measurements of Oral Hygiene Index-Simplified (OHI-S) Index by Green and Vermillion (1964) was recorded at baseline and after 1 month of scaling and root planing, whereas pocket probing depth was recorded using UNC-15 probe at the mesial, distal, buccal and palatal or lingual aspect of all teeth excluding third molar at baseline for diagnostic purpose.

Collection of blood (Figure 3a and 3b)



Figure 3a: Venous blood collection from antecubital fossa.



Figure 3b: Collected venous blood in the plain vial.

Under aseptic precautions, 2-3 ml venous blood was collected from subjects by a single venepuncture in the antecubital fossa in plain vials, at baseline and 1 month after non-surgical periodontal therapy in aggressive and chronic periodontitis whereas only at baseline in non-periodontitis group, which was sent for laboratory analysis. All biosafety measures were followed.

Hematological parameters to be evaluated

Serum C-reactive protein levels (CRP) was evaluated by a commercially available kit (Accucare CRP- turbilatex). WBC counting was done using cell counting machine (CELL DYN 1800).

Statistical analyses

Data is presented in form of frequency tables and descriptive statistics. The collected data was analysed using STATA IC-13 software. To compare two groups of mean, independent t-test was used. Paired t-test was used to check effect of intervention and Analysis of Variance (ANOVA) test was used to compare more than two groups of mean simultaneously. Post Hoc test (Tukey's test) was applied for mutual comparison. To check relationship between two variables, Pearson's correlation coefficient was obtained. For each statistical test, p-value was obtained and statistical significance was decided as 0.05 (5%).

Results

Total 57 subjects [21(36.84%) males and 36(63.16%) females] were taken for the study, out of which 17 (29.8%) were group A, 20 (35.1%) were group B and remaining 20 (35.1%) were group C. Among 57 subjects, 18 (31.6%) had good oral hygiene, 23 (40.4%) had fair oral hygiene and 16 (28.1%) had poor oral hygiene at baseline (Table 1).

		Total n=57 (100%)	Mean age	male	female
Sample (n)	Group A	17 (29.8%)	28.41 (5.43)	4 (23.53%)	13 (76.47%)
	Group B	20 (35.1%)	38.8 (7.7)	7 (35%)	13 (65%)
	Group C	20 (35.1%)	24.85 (4.3)	10 (50%)	10 (50%)
Age	<= 30	32 (56.1%)			
	31-40	16 (28.1%)			
	41-50	9 (15.8%)			

Sex	male	21 (36.8%)			
	female	36 (63.2%)			
Oral hygiene	good	18 (31.6%)			
	fair	23 (40.4%)			
Status (baseline)	poor	16 (28.1%)			

Table 1: Demographic details at baseline.

*Data represent mean \pm standard deviation (SD).

CRP

At baseline, mean CRP level of group A was 5.091 ± 10.02 whereas that of group B and group C was 2.658 ± 1.82 and 1.917 ± 0.79 respectively (Table 2). There was statistically no significant difference in mean CRP level between three groups (p -value = 0.213).

After one month, mean CRP level of group A was 3.0153 ± 3.37 whereas that of group B was 3.0030 ± 2.24 . There was statistically no

significant difference in mean CRP level between two groups (p -value=0.990).

WBC

At baseline, mean WBC level of group A was $1.03E4 \pm 1850.85$ whereas that of group B and group C was 9464.00 ± 1904.62 and 7510.00 ± 1263.20 respectively (Table 2).

	Group A (n= 17)			Group B (n=20)			Group C (n= 20)
	Baseline	After 1 month	P value	Baseline	After 1 month	P value	Baseline
CRP	5.09 (10.02)	3.01 (3.37)	$p > 0.05$	2.66 (1.82)	3.00 (2.25)	$p > 0.05$	1.92 (0.79)
WBC	10.3 (1.8)	7.9 (1.5)	$p < 0.001$	9.5 (1.9)	8.5 (1.8)	$p < 0.05$	7.5 (1.3)
OHI-S	2.88 (0.84)	0.70 (0.31)	$p < 0.001$	3.15 (0.88)	0.65 (0.40)	$p < 0.001$	0.78 (0.37)

Table 2: Changes in CRP, WBC and OHI-S at baseline and after 1 month of non-surgical periodontal therapy among three groups.

*Data represent mean \pm standard deviation (SD).

There was statistically a highly significant difference in mean WBC level between three groups (p -value= <0.001).

After one month, mean WBC level of group A was 7976.47 ± 1538.47 whereas that of group B was 8510.00 ± 1778.79 . There was statistically no significant difference in mean WBC level between two groups (p -value=0.340).

In group A, mean WBC level at baseline is $1.03E4 \pm 1850.85$ whereas it is 7976.47 ± 1538.47 after one month. There was statistically a highly significant difference in mean WBC level before and after scaling & root planing (p -value=0.001).

In group B, mean WBC level at baseline is 9464.00 ± 1904.62 whereas it is 8510.00 ± 1778.79 after one month. There was statistically significant difference in mean WBC level before and after scaling & root planing (p -value=0.013).

OHI-S

At baseline, mean OHI-S level of group A was 2.8847 ± 0.83 whereas that of group B and group C was 3.1480 ± 0.87 and 0.7815 ± 0.36 respectively (Table 2). There was statistically highly significant difference in mean OHI-S level between three groups (p -value= <0.001).

After one month, mean OHI-S level of group A was 0.7029 ± 0.30 whereas that of group B was 0.6465 ± 0.40 . There was statistically no significant difference in mean OHI-S level between two groups (p -value=0.638).

In group A, mean OHI-S level at baseline is 2.8847 ± 0.83 whereas it is 0.7029 ± 0.30 after one month. There was statistically highly significant difference in mean OHI-S level before and after scaling & root planing (p -value= <0.001).

In group B, mean OHI-S level at baseline is 3.1480 ± 0.87 whereas it is 0.6465 ± 0.40 after one month. There was statistically highly significant difference in mean OHI-S level before and after scaling & root planing (p -value= <0.001).

- There is a strong positive correlation between age of patients and OHI-S score at baseline (r -value=0.537, p -value <0.001) (Table 3). As the age increases, oral hygiene maintenance reduces.

- There is a positive correlation between WBC and OHI-S score at baseline (r -value=0.468, p -value <0.001) (Table 3). As oral hygiene maintenance worsens, WBC count increases.

- There is a negative correlation between WBC and OHI-S score at baseline (r -value=-0.369, p -value <0.05) (Table 3). As oral hygiene maintenance improves, WBC count decreases.

		OHI-SB
AGE	Pearson Correlation	0.537
	Sig. (2-tailed)	0.000
	N	57
WBCB	Pearson Correlation	0.468
	Sig. (2-tailed)	0.000
	N	57
WBCM	Pearson Correlation	-0.369
	Sig. (2-tailed)	0.025
	N	37

Table 3: Correlation between variables.

Discussion

Over the last decade, evidence has been accumulating that associates severe periodontitis with a higher propensity to form atherosclerotic plaques, which are responsible for myocardial infarction, ischemic stroke, and peripheral arterial disease [6]. Different pathogenetic mechanisms have been proposed in these associations, including the possible direct role of oral bacteria in the pathogenesis of atherosclerotic plaques and the involvement of systemic inflammatory mediators, such as CRP, triggered by periodontal infections [7,8].

Numerous studies have attested to the predictive value of CRP measurement for cardiovascular disease development and prognosis, some suggesting that CRP may be more powerful than traditional risk factors such as low-density lipoprotein (LDL) cholesterol. Several case-control studies have emphasized that patients with chronic destructive periodontal disease have increased serum CRP levels when compared with unaffected healthy control patients [7,9,10]. Separate studies have explored the potential effects of periodontal treatment on circulating CRP and other surrogate markers of the vascular response [3,11].

The meta-analysis of Paraskevas et al. [12] suggest that non-surgical periodontal therapy lowers the level of CRP in patients with periodontal disease, whereas meta-analysis of Ioannidou et al. [6] does not support the hypothesis that non-surgical periodontal therapy can reduce systemic CRP levels. Thus, in the present study we have compared and determined the effect of non-surgical periodontal therapy on serum CRP level in patients with aggressive and chronic periodontitis.

Due to higher variation in CRP levels in aggressive periodontitis group and relatively small sample size, there was statistically no significant difference seen in present study as compared to other studies [8,13].

Due to higher variation in CRP levels in chronic periodontitis group, there was no statistically significant difference seen in present study as compared to other studies [10,14].

In present study, serum CRP levels in chronic periodontitis group increased after non-surgical periodontal therapy as compared to baseline. The possible fact behind these findings: the mechanism of

this could be that both the bacteremia and the tissue damage following sub-gingival instrumentation [15,16]. Our results are in concert with the studies done by Ushida et al. [17] and Graziani et al. [18], which does not support the hypothesis that periodontal treatment can reduce systemic serum CRP levels. Moreover, there are few studies [19,20], which are in contrast to our results showing that non-surgical periodontal therapy lowers the level of serum CRP in patients with periodontal disease.

In present study, mean WBC levels in aggressive periodontitis group decreased significantly after non-surgical periodontal therapy as compared to baseline. Our result is consistent with the results of Christan et al. [21], in non-smokers group. The results of the present study indicate that non-surgical periodontal therapy may lead to a significant reduction of leukocyte counts in non-smoking systemically healthy patients. The leukocyte count has been demonstrated in several epidemiological studies to be an independent predictor of future coronary heart disease [22,23]. Thus, our results show that periodontal therapy has systemic effects and has the potential to ameliorate an established cardiovascular risk factor.

This study has certain limitations

1) CRP assaying was not done by high sensitivity CRP measurements, following a single course of periodontal treatment, residual diseased sites may have remained 2) there might have been insufficient time for biochemical changes to be established after disease reduction and 3) relatively small sample size.

Conclusion

Periodontitis has been linked to cardiovascular disease, although mechanisms for this association are obscure. Several parameters of systemic inflammation have been identified as markers of cardiovascular diseases. Thus, the present study was carried out to determine the effect of non-surgical periodontal therapy on serum CRP level in patients with aggressive and chronic periodontitis. Hence it was designed to explore further in understanding the role played by serum C-reactive protein in patients with aggressive periodontitis, chronic periodontitis and non-periodontitis.

Based on the findings of our present study, it is highly unlikely that CRP levels can be modulated by non-surgical periodontal treatment alone in patients with severe periodontal disease. Moreover, CRP being a non-specific marker of the acute-phase response many potential stimuli, including (unknown) chronic infections and or inflammatory conditions, smoking, obesity and trauma, may also account for mild increases in CRP. This finding further underscores the importance of conducting future well-designed, sufficiently powered, randomized controlled clinical studies to confirm whether attenuation of the systemic inflammatory process can alter the atherothrombotic process in patients with periodontal disease.

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