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Original Article

Evaluation of C-reactive Protein Level in Aggressive and Chronic Periodontitis Patients Before and After Phase 1 Therapy: A Case Control Study

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Abstract

Background: Many studies have been carried out till date that talks about the chronic periodontitis and aggressive periodontitis but none correlates with C-reactive protein (CRP) level in blood. Since CRP is an important biomarker for systemic inflammation, it must be interesting to check whether periodontal health status has anything to do with the CRP level in blood. Hence, the present study was carried out to determine CRP level in blood before and after instituting Phase I periodontal therapy and to find out whether it has any effect on systemic health as shown by the correlating CRP level.

Aim: The aim of the study was to determine and compare the levels of serum C-reactive protein before and after Phase I therapy in patients with chronic periodontitis and patients with aggressive periodontitis.

Materials and Methods: A total of 30 subjects were selected, 10 each of generalized chronic periodontitis, generalized aggressive periodontitis and non-periodontitis (control group). Blood sample was collected from all the subjects at baseline and 3 months after Phase I therapy. Clinical parameters such as plaque index, gingival bleeding index, probing pocket depth, and clinical attachment level were recorded at baseline and at 3 months after Phase I therapy.

Results: Significant reduction was observed in the serum CRP level in both chronic periodontitis and aggressive periodontitis patients 3 months after Phase I therapy as compared to baseline level while in non-periodontitis patients, no significant reduction was noted in serum CRP level 3 months after Phase I therapy as compared to baseline.

Conclusion: Within the limits of the present study, it can be concluded that both forms of periodontitis may play a role in systemic inflammation as indicated by elevated serum CRP level and more importantly, treating periodontal disease alone could bring down the inflammation as indicated by reduced serum CRP levels which help in minimizing the risk of systemic inflammation.

Key words: Periodontal therapy, Periodontitis, Phase I therapy, Serum C-reactive protein

INTRODUCTION

Periodontitis is one the most common inflammatory disease of the tooth-supporting structures which if left untreated will progress to alveolar bone destruction and tooth loss.[1] Advances in science and technology over the last three decades have greatly expanded our knowledge about the pathogenesis of periodontal diseases.[2] Periodontitis is an infectious disease associated with microorganisms, predominantly gram negative that exists in subgingival biofilm and individuals are not uniformly susceptible to periodontal diseases.[3]

The host responds to the periodontal infections with an array of events involving both innate and adaptive

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immunity. Although periodontitis is chronic in nature, acute phase elements are also part of the innate immunity in periodontitis and their presence confirms that in periodontitis, the systemic inflammation is present.[4,5] The acute phase reactant receiving the most attention is C-reactive protein (CRP) that serves as a systemic marker of inflammation[6] produced by the liver and concentration exceeding 10 mg/l is generally regarded as the threshold indicative significant inflammatory disease[7] which is generally low in 98% of the general population.

CRP possesses the ability to reveal inflammation at an early stage as it rises in serum within 48 hours. Its long plasma half-life of 12–18 h is constant under most of the conditions and hence that the sole determinant of circulating CRP is the synthesis rate,[8] which directly reflects the intensity of the pathological process stimulating CRP production. This property is useful for early detection of patients who are at risk for inflammatory disease.

The reason for interest in serum CRP level in periodontitis lies in the fact that periodontitis is associated with CVD.[9] The emergence of periodontal infections as a possible risk factor for cardiovascular disease is leading to convergence in oral and medical care. A number of studies have demonstrated an association between periodontal disease and the risk of myocardial infection and stroke as well as the underlying condition atherosclerosis.[10-12]

It is conceivable that elevated levels of CRP in both forms of periodontitis and decrease in their level after phase 1 therapy can explain partly an association between periodontitis and CVD and if a relationship exists between periodontal diseases and systemic CRP, it has a substantial clinical relevance in helping to explain circumstances in which an intraoral source of infection can create a systemic inflammatory response, therefore placing apparently healthy patients at increased risk of cardiovascular diseases.[13]

Chronic and aggressive forms of periodontitis show disparity in the rate of progression. Their effect on CRP levels seems to be an appealing area of research. Thus, the present study was undertaken to determine the relative levels of serum CRP and compare them in aggressive and chronic periodontitis patients and correlating the serum CRP levels with severity of the disease.

Aim and Objectives

The aim of the study was to determine and compare the levels of CRP in serum before and after Phase I therapy in

- Patients with chronic periodontitis
- Patients with aggressive periodontitis
- Non-periodontitis patients (control group).

MATERIALS AND METHODS

Study Design

Institution Ethical Committee issued the ethical clearance for the following protocol. The study was carried out in 30 subjects divided into three groups, with 10 subjects in each group:

Selected samples are from the outpatient section of the Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai.

Sample size: 30 (10 in each group)

Test Groups

Group I-CGP patients

Probing pocket depth (PPD) of ≥5 mm and/or clinical attachment loss (CAL) >30% sites with varying degree of disease severity.

Group II-GAP patients

≤30 years of age having probing depth (PD) of ≥5 mm and/or CAL on 8 or more teeth, at least three of which were not first molars and incisors with varying degree of disease severity.

Control Group

Group III – Non-periodontitis patients – PD ≤2 mm along with no evidence of attachment loss.

Inclusion Criteria

The following criteria were included in the study:

1. Patients with untreated chronic periodontitis
2. Patients with untreated aggressive periodontitis
3. Non-periodontitis patients with PD ≤2 mm along with no evidence of attachment loss.

Exclusion Criteria

The following criteria were excluded from the study:

1. Smokers and tobacco users
2. Patients with hematological disorders
3. Patients who are pregnant and lactating
4. Patients who are medically compromised (diabetes and other immunodeficiency syndromes, cardiovascular diseases, kidney, liver, and lung diseases)
5. Patients who were under systemic antibiotic therapy during the last 3 months
6. Patients who have undergone any sort of dental treatment under local anesthesia in the past 3 months
7. Patients with history or presence of any other chronic infections.

A thorough medical and dental history of the subjects was taken. All the subjects underwent full-mouth periodontal probing and charting and clinical and laboratory evaluation.

Method of Collecting Data

Clinical parameters assessment

The following clinical parameters were evaluated for all the subjects:

1. Plaque index (PI) – Silness and Loe, 1964[14]
2. Gingival bleeding index (GBI) – Ainamo and Bay, 1975[15]
3. PD in mm – Carranza 10th ed[16]
4. Clinical attachment level (CAL) in mm – Carranza 10th ed.[16]

Estimation of CRP

The subjects were informed and consent was taken. Three milliliters of venous blood were drawn from the antecubital vein of the participants. Samples were centrifuged in the centrifuge machine at 3000 rpm for 10 min to separate the serum from blood. Separated serum was collected in Eppendorf and stored in the deep freeze at –20°C. Quantitative determination of CRP in patient's serum was done by enzyme-linked immunosorbent assay (ELISA) method.

Principle of the Assay

Qualitative determination of CRP in patient blood was done by a double antibody sandwich ELISA method. In this assay, the CRP present in the sample reacts with anti-CRP antibodies which had been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound sample proteins by washing, anti-CRP antibodies conjugated with horseradish peroxidase proteins were added. These enzyme-labeled antibodies formed complexes with the previously bound CRP. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate 0,3'-8'5-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of CRP in the test sample.

The quantity of CRP in the test sample can be interpolated from the standard curve constructed from standard and corrected for serum dilution.

Reagents Used

1. Diluent concentration (running buffer) one bottle containing 50 ml of a ×5 conc. diluents running buffer
2. Wash solution concentrate: One bottle containing 50 ml of a ×20 concentrated wash solution
3. Enzyme antibody conjugate ×100: One vial containing 150 ul of affinity-purified anti-human CRP antibody conjugated with horseradish peroxidase in a stabilizing buffer
4. Chromogen substrate solution one vial containing 12 ml of 0,3'–8'5- TMB and hydrogen peroxide in citric buffer at pH 3.3
5. Stop solution one vial containing 12 ml of 0.3 M sulfuric acid
6. Anti-human CRP ELISA microplate 12 removable eight well Microwell Strips in well holder frame. Each well is coated with affinity-purified anti-human CRP
7. Human CRP calibrator. One vial containing a lyophilized human CRP calibrator.

Procedure

1. All reagents were brought to room temperature before use
2. Pipette 100 ul of
   - Standard 0 (0.0 ng/ml) in duplicate
   - Standard 1 (1.56 ng/ml) in duplicate
   - Standard 2 (3.125 ng/ml) in duplicate
   - Standard 3 (6.25 ng/ml) in duplicate
   - Standard 4 (12.5 ng/ml) in duplicate
   - Standard 5 (25 ng/ml) in duplicate
   - Standard 6 (50 ng/ml) in duplicate
   - Standard 7 (100 ng/ml) in duplicate.
3. Pipette 100 µl of serum sample in duplicate into predesignated wells
4. Incubate the microtiter plate at room temperature for 15 min (15 ± 2) min. Keep plate covered during incubation and following incubation, aspirate the contents of the well
5. Fill each well with appropriately diluted wash solution and aspirate. Repeat three times, for a total of four washes. Finally, invert the plate on absorbent paper (paper towel) and blot the excess fluid from the wells
6. Pipette 100 ul of the appropriately diluted enzyme-antibody conjugate to each well. Incubate at 22°C (room temperature) for 15 (15 ± 2) min
7. Wash and blot the wells as described in steps 5/6. Pipette 100 µl of TMB substrate solution into each well and incubate at room temperature for precisely 10 min
8. After 10 min, add 100 µl of stop solution to each well and determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to air.

Optical density (OD) of standard, control, and samples was read at 450 nm. Difference of OD was calculated, thereby plotting a standard curve and concentration of control, standard, and sampled were read and corrected for sera dilution.

RESULTS

In the present interventional study, among in Group I, II, and Group III. Five males and 5 females in each group, with a mean age of 39.40 ± 6.89 years in Group I, 23.90 ± 3.41 in Group II, and 35.00 ± 11.27

years in Group III. The parameters were assessed and recorded at baseline and 3 months postoperatively as follows,

PI

Intragroup comparison

Group I: The mean PI score at baseline was 2.48 ± 0.26 and at 3 months was 1.05 ± 0.23. The mean difference in plaque score from baseline to 3 months was statistically significant ($P = 0.032$) [Tables 1,2 and Figure 1].

Group II: The mean PI score at baseline was 2.20 ± 0.15 and at 3 months was 0.68 ± 0.16. The mean reduction in plaque score from baseline to 3 months was statistically significant ($P = 0.0001$).

Group III: The mean PI score at baseline was 0.61 ± 0.14 and at 3 months was 0.53 ± 0.09. The mean reduction in

Table 1: Comparison of plaque scores

Groups	Time duration (mean ±SD)		*P*-value
	Baseline	3 months post-operative	
Chronic periodontitis group	2.48±0.26	1.05±0.23	0.032*
Aggressive periodontitis group	2.19±0.15	0.68±0.16	0.0001*
Control group	0.61±0.14	0.53±0.09	0.122*
P-value	0.0001**	0.0001**	-

*Paired sample *t*-test, **One-way ANOVA

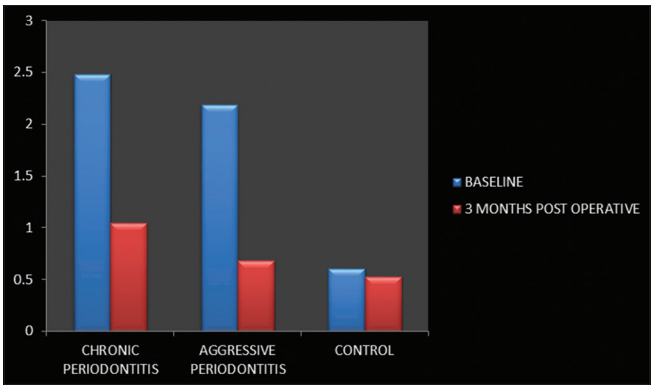

Figure 1: Comparison of plaque index between Group I, Group II, and Group III

Table 2: Individual comparison with Tukey post hoc- plaque score

			Tukey HSD				
Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Baseline plaque score	Chronic periodontitis group	Aggressive periodontitis group	0.28300*	0.08646	0.008	0.0686	0.4974
		Control group	1.87300*	0.08646	0.000	1.6586	2.0874
	Aggressive periodontitis group	Chronic periodontitis group	−0.28300*	0.08646	0.008	−0.4974	−0.0686
		Control group	1.59000*	0.08646	0.000	1.3756	1.8044
	Control group	Chronic periodontitis group	−1.87300*	0.08646	0.000	−2.0874	−1.6586
		Aggressive periodontitis group	−1.59000*	0.08646	0.000	−1.8044	−1.3756
Plaque score 3 months	Chronic periodontitis group	Aggressive periodontitis group	0.37200*	0.07493	0.000	0.1862	0.5578
		Control group	0.51800*	0.07493	0.000	0.3322	0.7038
	Aggressive periodontitis group	Chronic periodontitis group	−0.37200*	0.07493	0.000	−0.5578	−0.1862
		Control group	0.14600	0.07493	0.145	−0.0398	0.3318
	Control group	Chronic periodontitis group	−0.51800*	0.07493	0.000	−0.7038	−0.3322
		Aggressive periodontitis group	−0.14600	0.07493	0.145	−0.3318	0.0398

**P*-Value <0.05

Table 3: Individual comparison with Tukey *post hoc*- probing pocket depth

Dependent variable	(I) Groups	(J) Groups	Mean difference (I-J)	Std. Error	Sig.	95% Confidence interval	
						Lower Bound	Upper Bound
Baseline PPD	Chronic periodontitis group	Aggressive periodontitis group	0.39800	0.37577	0.547	–0.5337	1.3297
		Control group	2.11500*	0.37577	0.000	1.1833	3.0467
	Aggressive periodontitis group	Chronic periodontitis group	–0.39800	0.37577	0.547	–1.3297	0.5337
		Control group	1.71700*	0.37577	0.000	0.7853	2.6487
	Control group	Chronic periodontitis group	–2.11500*	0.37577	0.000	–3.0467	–1.1833
		Aggressive periodontitis group	–1.71700*	0.37577	0.000	–2.6487	–0.7853
PPD 3 months	Chronic periodontitis group	Aggressive periodontitis group	–0.10100	0.26222	0.922	–0.7512	0.5492
		Control group	0.58800	0.26222	0.082	–0.0622	1.2382
	Aggressive periodontitis group	Chronic periodontitis group	0.10100	0.26222	0.922	–.5492	0.7512
		Control group	0.68900*	0.26222	0.036	0.0388	1.3392
	Control group	Chronic periodontitis group	–0.58800	0.26222	0.082	–1.2382	0.0622
		Aggressive periodontitis group	–0.68900*	0.26222	0.036	–1.3392	–0.0388

**P*-Value <0.05

PI from baseline to 3 months was statistically insignificant ($P = 0.122$) [Table 3 and Figure 1].

Intergroup comparison

Mean difference between Group I and Group II at baseline was 0.283 which was statistically significant ($P = 0.008$) and at 3 months post-operative was 0.372 which was statistically significant ($P = 0.000$).

Mean difference between Group I and Group III at baseline was 0.518 which was statistically significant ($P = 0.000$) and at 3 months post-operative was 0.588 which was statistically significant ($P = 0.000$).

Mean difference between Group II and Group III at baseline was 1.590 which was statistically significant ($P = 0.000$) and at 3 months post-operative was 0.146 which was statistically non-significant ($P = 0.145$).

Table 4: Comparison of gingival bleeding index

Group	Time duration (frequency)				*P*-value
	Baseline		3 months		
	Absence	Presence	Absence	Presence	
Chronic periodontitis	0	10	10	0	0.0001**
Aggressive periodontitis	0	10	7	3	0.023**
Control	10	0	10	0	1.00**
P-value	0.0001*		0.036*		-

*Chi-square test, **McNemar test

Table 5: Comparison of probing pocket depth

Groups	Time duration (mean±SD)		*P*-value
	Baseline	3 months post-operative	
Chronic periodontitis group	4.27±1.31	2.71±0.83	0.0001*
Aggressive periodontitis group	3.87±0.62	2.82±0.567	0.0001*
Control group	2.15±0.16	2.13±0.16	0.606*
P-value	0.0001**	0.029**	-

*Paired sample *t*-test. **One-way ANOVA

GBI

Gingival bleeding score was 1 at all cases of Group I and II at baseline, negative (score 0) in all Group I and 7 cases of Group II, only 3 cases of Group II remains with score 1 after 3 months postoperatively. Group III cases present only with score 0 at baseline and after 3 months [Table 4 and Figure 2].

PPD

Intragroup comparison

Group I: The mean PPD at baseline was 4.27 ± 1.30 and at 3 months was 2.71 ± 0.82. The mean reduction in PPD from baseline to 3 months was statistically significant ($P = 0.0001$).

Group II: The mean PPD at baseline was 3.87 ± 0.62 and at 3 months was 2.82 ± 0.57. The mean reduction in PPD from baseline to 3 months was statistically significant ($P = 0.0001$) [Tables 3,5 and Figure 3].

Group III: The mean PPD at baseline was 2.16 ± 0.16 and at 3 months was 2.713 ± 0.16 the mean reduction in PPD

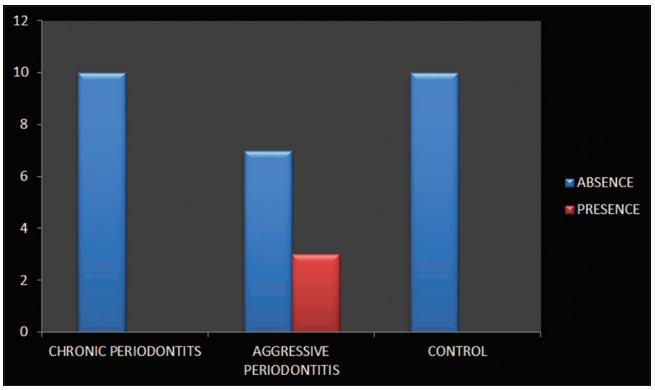

Figure 2: Comparison of a gingival bleeding index between Group I, Group II, and Group III

Table 6: Individual comparison with Tukey post hoc test-serum CRP level

Dependent variable	(I) Groups	(J) Groups	Mean difference (I-J)	Std. Error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
Baseline CRP	Chronic periodontitis group	Aggressive periodontitis group	1.61427*	0.57214	.023	.1957	3.0328
		Control group	4.09860*	0.57214	0.000	2.6800	5.5172
	Aggressive periodontitis group	Chronic periodontitis group	–1.61427*	0.57214	0.023	–3.0328	–0.1957
		Control group	2.48433*	0.57214	0.001	1.0658	3.9029
	Control group	Chronic periodontitis group	–4.09860*	0.57214	0.000	–5.5172	–2.6800
		Aggressive periodontitis group	–2.48433*	0.57214	0.001	–3.9029	–1.0658
CRP 3 months	Chronic periodontitis group	Aggressive periodontitis group	.65460	0.47539	0.367	–0.5241	1.8333
		Control group	1.77650*	0.47539	0.002	0.5978	2.9552
	Aggressive periodontitis group	Chronic periodontitis group	–.65460	0.47539	0.367	–1.8333	0.5241
		Control group	1.12190	0.47539	0.064	–0.0568	2.3006
	Control group	Chronic periodontitis group	–1.77650*	0.47539	0.002	–2.9552	–0.5978
		Aggressive periodontitis group	–1.12190	0.47539	0.064	–2.3006	0.0568

**P*-Value <0.05

from baseline to 3 months was statistically non-significant ($P = 0.606$) [Table 6 and Figure 3].

Intergroup comparison

Mean difference between Group I and Group II at baseline was 0.398 which was statistically non-significant ($P = 0.547$) and at 3 months post-operative was −0.101 which was statistically non-significant ($P = 0.922$).

Mean difference between Group I and Group III at baseline was 2.115 which was statistically significant ($P = 0.000$) and at 3 months post-operative was 0.588 which was statistically non-significant ($P = 0.082$).

Mean difference between Group II and Group III at baseline was 1.717 which was statistically significant ($P = 0.000$) and at 3 months post-operative was 0.689 which was statistically significant ($P = 0.036$).

CAL

Intragroup comparison

Group I: The mean CAL at baseline was 4.00 ± 1.33 and at 3 months was 3.06 ± 1.00. The mean reduction in CAL from baseline to 3 months was statistically significant ($P = 0.032$) [Tables 7,8 and Figure 4].

Group II: The mean CAL at baseline was 3.98 ± 0.67 and at 3 months was 2.97 ± 0.54. The mean reduction in CAL from baseline to 3 months was statistically significant ($P = 0.0001$).

Group III: There was no CAL either at baseline or 3 months post-operative.

Intergroup comparison

Mean difference between Group I and Group II at baseline was 0.024 which was statistically non-significant ($P = 0.998$) and at 3 months post-operative

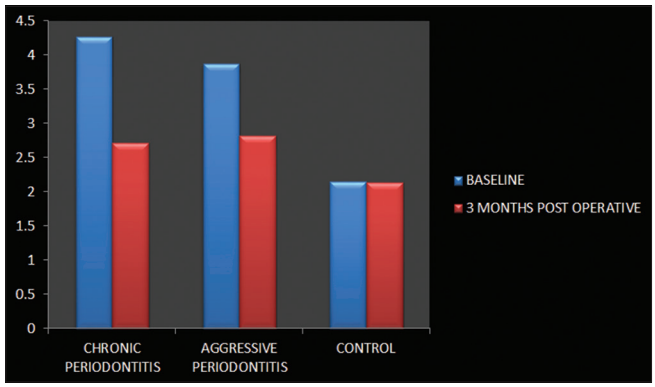

Figure 3: Comparison of probing pocket depth between Group I, Group II, and Group III

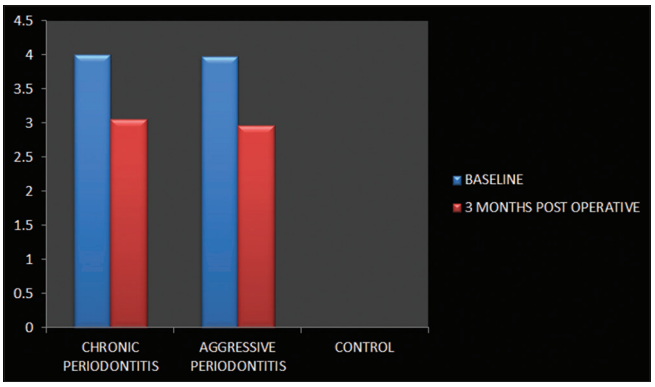

Figure 4: Comparison of clinical attachment level between Group I, Group II, and Group III

Table 7: Comparison of clinical attachment loss

Groups	Time duration (mean±SD)		*P*-value
	Baseline	3 months post-operative	
Chronic periodontitis group	4.00±1.33	3.06±1.01	0.032*
Aggressive periodontitis group	3.98±0.67	2.97±0.55	0.0001*
Control group	0.00±0.00	0.00±0.00	***
P-value	0.0001**	0.0001**	-

*Paired sample *t*-test, **One-way ANOVA

Table 8: Individual comparison with Tukey post hoc test-clinical attachment loss

Dependent variable	(I) Groups	(J) Groups	Mean difference (I-J)	Std. Error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
Baseline CAL	Chronic periodontitis group	Aggressive periodontitis group	0.02400	0.38529	0.998	–0.9313	0.9793
		Control group	4.00400*	0.38529	0.000	3.0487	4.9593
	Aggressive periodontitis group	Chronic periodontitis group	–0.02400	0.38529	0.998	–0.9793	0.9313
		Control group	3.98000*	0.38529	0.000	3.0247	4.9353
	Control group	Chronic periodontitis group	–4.00400*	0.38529	0.000	–4.9593	–3.0487
		Aggressive periodontitis group	–3.98000*	0.38529	0.000	–4.9353	–3.0247
CAL 3 months	Chronic periodontitis group	Aggressive periodontitis group	0.09700	0.29647	0.943	–0.6381	0.8321
		Control group	3.06800*	0.29647	0.000	2.3329	3.8031
	Aggressive periodontitis group	Chronic periodontitis group	–0.09700	0.29647	0.943	–0.8321	0.6381
		Control group	2.97100*	0.29647	0.000	2.2359	3.7061
	Control group	Chronic periodontitis group	–3.06800*	0.29647	0.000	–3.8031	–2.3329
		Aggressive periodontitis group	–2.97100*	0.29647	0.000	–3.7061	–2.2359

**P*-Value <0.05

Table 9: Comparison of serum CRP level

Groups	Time duration (mean±SD)		*P*-value
	Baseline	3 months post-operative	
Chronic periodontitis group	4.93±1.69	2.57±1.33	0.0001*
Aggressive periodontitis group	3.32±1.38	1.91±1.20	0.0001*
Control group	0.83±0.39	0.79±0.40	0.286*
P-value	0.0001**	0.003**	-

*Paired sample *t*-test, **One-way ANOVA

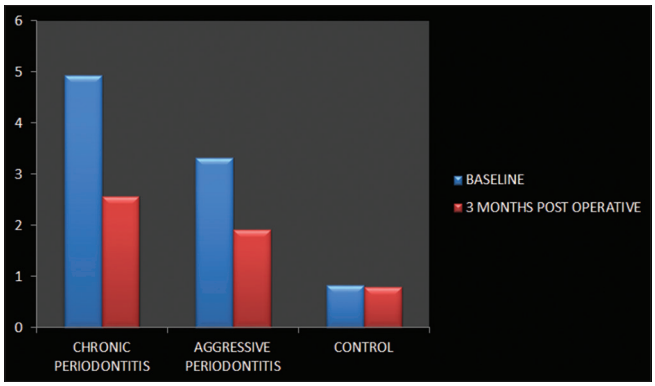

Figure 5: Comparison of serum CRP between Group I, Group II, and Group III

was 0.097 which was statistically non-significant (*P* = 0.943).

Mean difference between Group I and Group III was 4.00 which was statistically significant (*P* = 0.00) and at 3 months post-operative was 3.068 which was statistically significant (*P* = 0.000).

Mean difference between Group II and Group III was 3.98 which was statistically significant (*P* = 0.00) and at 3 months post-operative was 2.971 which was statistically non-significant (*P* = 0.000).

Serum CRP

Intragroup comparison

Group I: The mean serum CRP at baseline was 4.93 ± 1.67 and at 3 months was 2.57 ± 1.39. The mean reduction in serum CRP from baseline to 3 months was statistically significant (*P* = 0.0001) [Tables 6,9 and Figure 5].

Group II: The mean serum CRP at baseline was 3.32 ± 1.38 and at 3 months was 1.91 ± 1.20. The mean reduction in serum CRP from baseline to 3 months was statistically significant (*P* = 0.0001).

Group III: The mean serum CRP at baseline was 0.83 ± 0.40 and at 3 months was 0.79 ± 0.4. The mean reduction in serum CRP from baseline to 3 months was statistically non-significant (*P* = 0.286).

Intergroup comparison

Mean difference between Group I and Group II at baseline was 1.61 which was statistically significant (*P* = 0.023) and at 3 months post-operative was 0.654 which was statistically non-significant (*P* = 0.367).

Mean difference between Group I and Group III at baseline was 4.098 which was statistically significant (*P* = 0.000) and at 3 months post-operative was 1.776 which was statistically non-significant (*P* = 0.002).

Mean difference between Group II and Group III at baseline was 2.484 which was statistically significant (*P* = 0.001) and at 3 months post-operative was 1.121 which was statistically non-significant (*P* = 0.064).

DISCUSSION

Periodontitis is a progressive inflammatory disease of the supporting tissues surrounding the teeth caused by specific microorganisms.[1] Periodontitis is broadly classified into two groups; chronic periodontitis and aggressive periodontitis. Both forms represent the same disease with varying nature of disease severity and rate of progression. Earlier considered simply as a localized infection confined to the oral cavity, a growing body of evidence suggests that the pathology of periodontitis may affect the outcome of several systemic diseases, such as myocardial infarction, stroke, or preterm low birth weight babies.[17] The host responds to periodontal infections with an array of events involving both innate and adaptive immunity. This response in the periodontium can influence the systemic levels of many inflammatory mediators and acute-phase proteins. The acute phase reactant receiving the most attention is CRP. CRP has been the focus of attention as a key marker of acute-phase response to inflammation and its elevated levels constitute a risk predictor for cardiovascular disease.[5] As a consequence of its kinetics, it best describes the inflammatory status of an individual.[18]

Studies have indicated that serum CRP is elevated in patients with periodontal diseases (aggressive and chronic)[19] and treatment of periodontal infection significantly lowers the serum CRP levels whereas local factors such as pulp vitality,[20] excessive occlusal forces[21,22] also can influence the healing of periodontium after therapy. Hence, the local factors should be identified and eliminate at the initial stage itself.

The present study was carried out to compare the serum levels of CRP in patients of chronic and aggressive patients at the baseline level and 3 months after phase 1 therapy.

Several studies[13,23-26] have been carried out that have evaluated CRP level in saliva, serum, or GCF in patients of chronic periodontitis and aggressive periodontitis individually but very few have compared the serum CRP level before and after Phase 1 therapy in both forms of periodontitis.

In the present study, subjects with any acute or chronic systemic conditions such as diabetes or inflammatory conditions such as rheumatoid arthritis and cardiovascular disease have been excluded because these conditions can cause increased CRP level on their own, which may lead to confounding effect in the study.[26] Smoking is also a potential confounding factor because it is responsible for increases in CRP levels and is the principal environmental risk factor for periodontitis[27] and hence smokers were also excluded from the study.

Patients under medications such as antibiotics, corticosteroids, and anti-inflammatory drugs for past 3 months and those underwent periodontal therapy within past 6 months have been excluded because these therapies can suppress the inflammatory process and may lead to confounding effect in the study.

Quantitative determination of CRP in the present study in all the groups was done by double antibody sandwich ELISA method which is a very sensitive method for detecting CRP as compared to other methods.

In the present study, clinical parameters such as PI, PPD, CAL, and GBI were also assessed and compared among the three groups to correlate with the levels of serum CRP to establish a relationship between altered CRP levels and periodontal disease status.

In present study, PI, PPD, and CAL values at baseline in Group I (2.48 ± 0.26, 4.27 ± 1.30, and 4.00 ± 1.33, respectively) and in Group II (2.20 ± 0.15, 3.87 ± 0.62, and 3.98 ± 0.67, respectively) and in Group III were (0.61 ± 0.14, 2.16 ± 0.16, and 0.00). Were compared and the difference was highly significant.

The present study showed a significantly higher level of serum CRP in Group I (4.93 ± 1.67) and Group II (2.57 ± 1.39) as compared to Group III (0.83 ± 0.40) at the baseline level. The results of the present study indicate a significant correlation between PI, PPD, CAL, and CRP which is consistent with the findings of Noack *et al.*[25] who observed a statistically significant increase in CRP levels in 174 subjects with periodontal disease (4.06 ± 5.55 vs. 1.70 ± 1.91 mg/l) and a positive correlation between elevated levels of CRP and PI, PPD, and CAL. They found increased CRP levels in deeper pockets which could be due to the presence of periodontal Gram-negative pathogens like *Porphyromonas gingivalis* in the subgingival region.

In this study, it was found that the baseline CRP level in GCP and GAP was 4.93 and 3.32 mg/l, respectively and in the non-periodontitis control group at 0.83 mg/L which is in accordance with the study conducted by Ebersole and Cappelli (1997)[23] who detected that CRP levels were significantly increased in serum of adult periodontitis patients with CRP Levels at 9.12 mg/L versus 2.17 mg/L in healthy controls.

There are very few studies that have evaluated CRP levels in aggressive periodontitis subjects. One study by Salzberg *et al.*[28] reported an increase in CRP levels in generalized aggressive periodontitis patients (3.72 mg/L). The present study found similar CRP values (3.32 mg/l) in GAP patients at baseline.

After performing phase 1 therapy in all the patients, a significant reduction was found in serum CRP levels in chronic periodontitis and aggressive periodontitis patients (2.57 ± 1.39 and 1.91 ± 1.20, respectively) when compared to their baseline levels. This was in accordance with work done by D'Aiuto *et al.* (2004)[29] who found that systemically healthy subjects suffering from severe generalized periodontitis had higher CRP associated CVD risk and after receiving non-surgical periodontal therapy, the inflammatory level and risk of systemic diseases had reduced considerably. Whereas in non-periodontitis patients, serum CRP levels (0.79 ± 0.4) were found to be similar to their baseline levels.

Kumar *et al.*[30] also mentions that CRP levels strongly and independently predict the risk of myocardial infarction, stroke, peripheral artery disease, and sudden cardiac death, even among apparently healthy individuals. PI (1.05 ± 0.23 and 0.68 ± 0.16), PPD (2.71 ± 0.82 and 2.82 ± 0.57), and CAL (3.07 ± 1.00 and 2.97 ± 0.54) also showed a significant reduction in chronic and aggressive periodontitis patients at 3 months after phase 1 therapy when compared to their baseline values. Reduction in the values of the above mentioned clinical parameters exhibits a positive correlation with serum CRP level.

This is in accordance with work done by Santosh *et al.* (2013)[31] who found a 37% reduction in PPD and 45% gain in CAL and a reduction of about 90% reduction of CRP levels in the gingival crevicular fluid after 45 days.

Nakajima *et al.* (2010)[32] also confirmed that chronic periodontitis had high CRP level and subsequent periodontal treatment decreased the serum levels of CRP.

The present study was aimed at determining whether CRP is raised in periodontitis patients who are systemically healthy and if phase 1 therapy in these patients reduces inflammation which can reduce the risk of systemic inflammatory diseases like CVD. This is reflected in the reduction of CRP values at 3 months after phase 1 therapy as compared to baseline values. There are studies which show that patients with periodontitis had increased risk of CVD such as one done by De Stefano (1993)[33] which reported that patients with periodontitis had a 25% increased risk of coronary heart disease relative to those with minimal periodontal disease. Rosenberger *et al.*, 1996[34] stated that subjects with severe periodontitis had 4.3 times higher risk for cerebral ischemia than subjects with mild or without periodontal disease.

CONCLUSION

The following conclusions were drawn from the study:

- The serum CRP level is raised above normal values in both chronic and aggressive periodontitis
- In non-periodontitis patients, serum CRP level is only slightly raised above normal levels and is very less when compared to chronic and aggressive periodontitis
- After carrying out phase 1 therapy in all the groups, serum CRP levels show an appreciable reduction in chronic and aggressive periodontitis while in the non-periodontitis group, serum CRP levels do not show much change from baseline values.

Within the limits of our study, it can be concluded that both forms of periodontitis may play a role in systemic inflammation as indicated by elevated serum CRP level and more importantly, treating periodontal disease alone could bring down the inflammation as indicated by reduced serum CRP levels which help in minimizing the risk of systemic inflammation.

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