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Assessment and Comparison of Levels of Serum C- reactive Protein in Periodontally Diseased and Healthy Subjects: A Case Control Study

Dr. Shruti Gupta[1], Dr. Ira Gupta[2], Dr. Neelam Das[3], Dr. Rohit Gupta[4], Dr. Roopa D.A[5] and Dr. Janardhana Amaranath B.J [6]

[1, 4] *Reader, Department of Periodontology, Rama Dental college Hospital & Research Centre, Kanpur (UP)*

[2, 5, 6] *Professor, Department of Periodontology, Rama Dental college Hospital & Research Centre, Kanpur (UP)*

Abstract

Aims and Objectives: C-reactive protein is an acute-phase-reactant primarily produced by the liver in response to infection or trauma. Recent studies have demonstrated a correlation between periodontitis and elevated CRP levels. This study aims to compare the serum-CRP level in chronic and aggressive periodontitis patients with healthy controls.

Materials and Method: A case-control clinical study was conducted with a total of 75 systemically healthy subjects, where 25 subjects were selected in each groups: Group I, Healthy control subjects; Group II, generalized chronic periodontitis patients and Group III, generalized aggressive periodontitis patients. Serum-CRP levels were quantified by using Turbid metric immuno-assay kit at baseline. Kit used was "TURBILYTE-CRP" (Tulip diagnostics, Goa, India).

Results: Mean serum CRP levels were significantly higher in both GCP and GAP group as compared to control group at baseline. On comparing the clinical parameters at baseline for GCP & GAP group with control group values, mean score of serum CRP levels for GAP group was statistically significant ($P<0.001$) in comparison to GCP group, which in turn was statistically significant as compared to control group.

Conclusion: The present study indicates a positive correlation between CRP and periodontal disease severity with particular concern in younger individuals, where it could be a possible underlying pathway in the association between periodontal disease and risk for cardiovascular disease in periodontitis patients.

Keywords: Aggressive periodontitis, C - reactive protein, chronic periodontitis, nonsurgical therapy..

Introduction

Periodontitis defined as "an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both."[1]. Periodontal tissue infiltrated by lymphocytes, polymorph nuclear neutrophils (PMNs), plasma cells & macrophages like different types of inflammatory cells showing changes in systemic and local immuno-inflammatory response. Due to release of various inflammatory cells, cytokines e.g. IL-1, PGE2 & TNF-α are released which cause periodontal tissue destruction & loss of alveolar bone. As the sequelae of these systemic & local immuno-inflammatory reactions, cytokines are produced which increased of C-reactive protein production.[2]

In the periodontal disease patients, reduction in gingival inflammation, pocket depth & attachment loss is achieved by periodontal treatment. In non surgical periodontal therapy, microbial bio film, plaque and calculus are removed which decreased bacterial load and restrict ongoing disease process. [3, 4] C - reactive protein is a nonspecific protein, produced in liver as acute phase reactant in response to various pathological conditions. The level of CRP in healthy individuals is normally i.e. <0.3 mg/l. [5,6,7] Many studies stated that chronic periodontitis favors as a known risk factor for cardiovascular events., atherosclerosis & preterm low birth weight infant.[8,9] Micro-organisms associated with pathogenic periodontitis, in combination with other different risk factors of atherosclerosis, make a niche on the atherosclerotic heart valves.[10-13]

Based on the report of American Heart Association (AHA) and the Center for Diseases Control (CDC), individuals with serum CRP levels >3.0 mg/liter are

at high risk for future cardiovascular disease.[14] Third National Health and Nutrition Examination survey (NHANES III), observed a positive interlink among serum CRP level, periodontal disease and atherosclerotic complication. Thus, the periodontal disease can be considered as a risk factor for atherosclerosis and cardiovascular events.[15]

According to Tonetti MS et al., biomarkers may play a role in the diagnosis of periodontitis at the early stage and provide an improved assessment in severity of periodontitis. Serum CRP levels not only aid both in staging and grading of periodontitis but also considers the rate of progression of periodontitis. Grade A periodontitis (slow rate of progression) has CRP ≤ 1mg/L, grade B (moderate rate of progression) 1 to 3mg/L and grade C (rapid rate of progression)> 3mg/L.[16]

Studies [5, 17, 18, 19, and 20] have demonstrated levels of serum CRP and their comparison in aggressive and/or chronic periodontitis patients with healthy controls are comparatively few in literature. Hence, this study has been carried out with the aim to compare the serum-CRP level in untreated cases of chronic and aggressive periodontitis patients with healthy controls.

Materials and Method

The subjects for the study were selected from the OPD of Rama Dental College, Hospital and Research center, Kanpur. The informed consent was taken from each patient and the ethical clearance was taken by the ethical committee of Rama Dental College. Sample size was calculated on the basis of maximum and minimum variation in CRP among the three study groups using the formula for comparison of means discussed in 'A. Indrayan, Basic Methods of Medical Research' by putting SD's of CRP as 0.13 of minimum variation and 0.58 for maximum variation in cases and control and a difference of 0.4 considered to be clinically significant.Considering 95% confidence level, 80% power of study and 10% loss to follow up the sample size was calculated to be 25 for each group.

Diagnosis of the periodontal diseases was based on the detailed medical and dental history along with intraoral periapical radiograph of each patient.

Group I: Healthy Control group (C)

Periodontally healthy subjects, probing depth (PD) ≤ 3 mm and no evidence of clinical attachment loss

Group II: GCP group

Moderate to severe chronic periodontitis patients. Patient's age 30-50yrs, at least 8 teeth with PD ≥ 3mm, CAL ≥ 3mm and presence of alveolar bone loss (ABL) radio graphically.

Group III: GAP group

25 subjects diagnosed with generalized aggressive periodontitis. Patient's age 20 to 30 years having probing depth (PD) of ≥3 mm and/or CAL ≥3 on 8 or more teeth, at least three of which were not first molars and incisors with varying degree of attachment loss and deposits being inconsistent with periodontal destruction .

Inclusion criteria

- ✓ Males and Females, having a minimum of 20 teeth.
- ✓ Age between 20-50yrs.
- ✓ Systemically healthy individuals.

Exclusion criteria

- ✓ Pregnant and lactating females.
- ✓ Any systemic disease
- ✓ Patient under any medication for the past 3 months
- ✓ Subjects having any dental treatment in past 6 months
- ✓ High blood pressure, sleep disturbances, depression, excessive alcohol use, and smoking recently or in past 10 years.

Clinical procedure and study design Phase I

Plaque index [21] (PI) (LOE & SILNESS), Gingival index [22] (GI) (LOE &SILNESS), Bleeding index [4] (BI) (AINAMO & BAY), Calculus index [23] (CI) (NIDR), Probing depth, and Clinical attachment level were recorded at baseline. The probing depth (PD) and clinical attachment level (CAL) was evaluated using UNC-15 probe at 6 sites per tooth that is the mesiobuccal, distobuccal, midbuccal, mesiolingual, distolingual, midlingual by single examiner. Blood samples were collected from each participant at a point of time for estimation of serum CRP levels.

CRP protein estimation

Sample Collection

Venous blood was withdrawn from the participants selected for the study. The subjects were informed, and written consent was taken. They were made to tighten a fist so that vein was more palpable, and antecubital vein was selected for venipuncture. A tourniquet was applied about 1-2 inches above the antecubital fossa.[20] [Figure.1]

After cleansing the puncture site with 10% iso propanol solution, blood was withdrawn using a syringe with 24 gauge needles. Tourniquet was released and the blood flow restored. After drawing 3 mL of blood, sterile cotton ball was placed on the puncture site and needle was withdrawn. The subjects were instructed to apply mild finger pressure on the site for few minutes to avoid oozing out of blood.[20]

Samples were centrifuged in the centrifuge machine at 3000 rpm for 10 min to separate the serum from blood. [Figure. 2] Separated serum was collected and stored in the deep freeze at - 20°C.[20] Serum levels of serum CRP were quantified using turbid metric immunoassay according to the manufacturer's instructions. Kit used was "TURBILYTE-CRP" (Tulip diagnostics, Goa, India). [24] [Figure. 3]

Kit Content [24]

(R1) Activation Buffer - 0.1 % Sodium azide (NaN_3)
(R2) Anti-CRP Reagent - Ready to use uniform suspension of polystyrene latex particles coated with anti- CRP antibody.
(S) -Lyophilized CRP Calibrator - A lyophilized preparation of serum equivalent to the stated amount of CRP on mg/dl basis, when hydrated appropriately. The TURBILYTE-CRP™ calibrator is traceable to the W.H.O. International Reference Standard (85/506) for Human C-reactive protein.[24]

Principle of the high Sensitive CRP Assay [25]

The "TURBILYTE-CRP" (Tulip diagnostics, Goa, India) is a quantitative turbid metric test for the measurement of C-reactive protein (CRP) in human serum or plasma. It is based on the principle of agglutination reaction. The test specimen was mixed with activation buffer (R1) and TURBILYTE-CRP™ latex reagent (R2) and allowed to react. Presence of CRP in the test specimen resulted in the formation of an insoluble complex producing a turbidity, which was measured at 546 nm wavelength. The increase in turbidity corresponded to the concentration of CRP in the test specimen.

Procedure [25]

CRP examination was done manually by immune turbidimetry method using turbilyte kit in semi auto analyzer Erba Chem-5 plus. Reagents of kit were stored at 2-8℃. for serum CRP level estimation, firstly the 450 microliters of reagent 1 (R1) was added into a empty test tube with the help of micropipette. [Figure.4] after this, 50 microliter reagent 2 (R2) was added in the same test tube by micropipette. [Figure.5] Both the reagents were mixed well and incubated for 5 minutes at 37 ºC. Then the 3µL of the serum sample was added in the same test tube and the CRP level was measured at 546 nm in the semi auto analyzer. [Figure.6] Calibrator provided with the kit can be used to standardize the reagents when necessary.

Reference Value [25]

The reference value in normal population is ≤ 0.6 mg/dl.

Statistical Analysis

Statistical package for the social sciences (SPSS) version 18 was used for all the statistical analysis. A p-value of <0.05 was considered statistically significant. Pearson correlation was used to find the association of periodontal parameters with the changes of serum CRP levels for group II & III from the baseline.

Results

75 subjects (35 male and 40 female), 20-50yrs age participated in the study. The mean ages for healthy, GCP & GAP group were 26.08, 36.64 and 23.92yrs, respectively.

In intergroup comparison mean score of plaque index (PI), calculus index (CI), gingival index (GI) & bleeding index (BI) at base line for GCP group was statistically significant as compared to GAP group. Mean score of serum CRP levels, probing depth (PD) & clinical attachment level (CAL) for GAP group is statistically significant (P<0.001) in comparison to GCP which in turn was statistically significant as compared to control group. [TABLE 1]

On correlating the relation between all clinical parameter for control, GCP & GAP at baseline, PD & serum CRP levels had moderate positive linear relationship in GAP group and a weak positive relationship for GCP groups. [TABLE 2]

Discussion

The present study was conducted to compare the serum-CRP level in untreated cases of chronic and aggressive periodontitis patients with healthy controls. The result of this study showed an increase in serum CRP levels in GCP and GAP subjects as compared to the healthy controls, which approves the results of the previous studies. [20, 26]

In a study, Serum CRP levels was found elevated in localized & generalized aggressive periodontitis cases as comparison with non-periodontitis individuals.[5] it showed due to the

aggressive nature of disease, serum CRP levels is increased in mild cases of aggressive periodontitis. In current study, a positive linear relationship for serum CRP levels to PD & CAL was found in both GCP and GAP groups. Previous studies [27- 31] are also supported same findings. Acute phase reactant i.e. C- reactive protein production are elevated with release of cytokines, as a result of altered systemic host immune response in correlation with Sub gingival microorganisms, associated with periodontal infections.

Slade et al.[15] explained a dose- response relationship between C- reactive protein levels and disease severity of periodontitis. Similarly, present study also shows positive association between the serum CRP levels and moderate to severe cases of periodontitis. Thus, periodontal disease is contributing as a risk factor for various systemic inflammatory diseases such as atherosclerosis etc.

In the present study, the GCP & GAP group showed a significant improvement in various clinical parameters at baseline along with decrease in serum CRP values. These findings were confirmed in the previous studies [22, 32, and 33] which showed a significant reduction in serum CRP levels at baseline. Therefore, it could be explained that the periodontal infection raised the serum CRP levels in periodontitis patients that could be a risk factor for various systemic and local inflammation. Thus, association of serum CRP levels with periodontal disease severity is found in many studies, therefore in future more controlled and long-term studies are require. This correlation has been also useful in early diagnosis and detection of many diseases. Major limitation of this study is short duration and small sample size. Sometimes there may be inclusion of undiagnosed systemic risk factors that could also affect the results. Results of current study summarized that periodontal disease have a positive association with increased serum CRP levels and future periodontal treatment helps to reduce the serum levels of C-reactive protein and minimize local and systemic bacterial load. Thus, these findings suggest the possible relationship among serum CRP levels, periodontitis and risk factor for cardiovascular events, systemic inflammation and atherosclerotic lesion and also predicts that the complete periodontal therapy in periodontitis patients would be highly helpful for reduction of serum CRP, thus reducing systemic risks.

References

1. Armitage GC. Ann Periodontology. 1999; 4(1): 1-6.
2. Smitha CN, Soni S, BASU SK. Role of C- reactive protein and periodontal disease in systemic health: A review. J Adv Dent Res. 2011; 2(1): 1-6.
3. Perry DA, Schmid MO, Takei HN. Phase I periodontal therapy. In Newman MG, Takei H, Klokkevoid P, Carranza FA editors. Carranza's Clinical Periodontology. 10th ed. Saunders, Elsevier, St Louis, Missouri: 2006. p. 722-27.
4. Kanaparthy A, Kanaparthy R, Niranjan. Evaluation of serum C-reactive protein levels in subjects with aggressive and chronic periodontitis and comparison with healthy controls. Dent Res J 2012; 9(3): 261-5.
5. Salzberg TN et al. C reactive protein in patients with aggressive periodontitis. J Periodontol 2006;77: 933-9.
6. Aiuto F, Ready D, Tonetti MS. Periodontal disease and C-reactive protein-associated cardiovascular risk. J Periodontal Res 2004; 39: 236-41.
7. Thakare KS, Deo V, Bhongade ML. Evaluation of the C-reactive protein serum levels in periodontitis patients with or without atherosclerosis. Indian J Dent Res 2010; 21: 326-9.
8. Williams RC, Offenbacher S. Periodontal medicine: The emergence of a new branch of periodontology. Periodontol 2000; 23: 9-12.
9. Radafshar G et al. Association between Periodontal Disease and Elevated C - reactive protein in Acute Myocardial Infarction Patients. Journal of Dentistry, Tehran University of Medical Sciences 2006; 3(3): 127-4.
10. Ross R. Atherosclerosis – An inflammatory disease. N Engl J Med 1999; 340: 115-26.
11. Pejcic A, Kesic L, Milasin J. Association between periodontopathogens and CRP levels in patients with periodontitis in Serbia. J Dent Res Dent Clin Dent Prospects 2011; 5: 10-6.
12. Page RC. The role of systemic inflammatory mediators in the pathogenesis of periodontal disease. J Periodontol Res 1991; 26: 230-42.
13. Senthil N, Vandana KL, Sangeeta D. A study to qualitatively evaluate the serum levels of C-reactive protein (CRP) in various inflammatory periodontal diseases. J Indian Soc Periodontol 2001; 5: 27-30.
14. Kluft C, de Maat MP. Determination of the habitual low blood level of C-reactive protein in individuals. Ital Heart J 2001; 2: 172-80.
15. 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; 79(1): 49-57.
16. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Periodontol 2018; 89(1): 159– 72.
17. Ionnaidu E. Effect of periodontal treatment on serum C reactive protein: A systematic review and meta-analysis. J periodontal 2006; 77: 1635-42.
18. Mysak J. C-reactive protein in patients with aggressive periodontitis. J Dent Sci 2017; 12: 368-74.
19. Bansal T, Pandey A, Deepa D, Asthana AK. C-reactive protein (CRP) and its association with periodontal disease: a brief review. J Clin Diag Res 2014;8(70):21–4.
20. Goyal L, Bey A, Gupta ND, Sharma VK. Comparative evaluation of serum C-reactive protein levels in chronic and aggressive periodontitis patients and

association with periodontal disease severity. Contemp Clin Dent 2014; 5(4):484–8.
21. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condtion. Acta Odontol Scand 1964; 22:121-35.
22. Loe H. The Gingival Index, the Plaque Index and the Retention Index Systems. J periodontal 1967; 38(6):610-6.
23. Peter S. Essentials of preventive community dentistry. 3rded. NewDelhi: Arya publication; 2008.
24. Material safety data sheet, (According to 2001/58/EC), literature by turbilyte diagnostics (p) ltd.
25. Literature of turbilyte of CRP kit, by tulip diagnostics (p) ltd.
26. Desvarieux M. Periodontal microbiota and carotid intima-media thickness: the Oral Infections and Vascular Disease Epidemiology Study (INVEST). Circulation 2005; 111:576–82.
27. Iwamoto Y, Nishimura F, Soga Y. Antimicrobial periodontal treatment decreases serum C-reactive protein, tumor necrosis factor-alpha, but not adiponectin levels in patients with chronic periodontitis. J Periodontol 2003; 74:1231-6.
28. Offenbacher S, Beck JD, Moss K. Results from the Periodontitis and Vascular Events (PAVE) Study: a pilot multicentered, randomized, controlled trial to study effects of periodontal therapy in a secondary prevention model of cardiovascular disease. J Periodontol 2009; 80:190– 201.
29. Chopra R, Patil SR, Kalburgi NB. Association between alveolar bone loss and serum C-reactive protein levels in aggressive and chronic periodontitis patients. J Indian Soc Periodontol 2012; 16(1):28-1.
30. Pejcic Ana P. The Markers of Systemic Inflammation in Patients with Chronic Periodontitis: Leukocytes, C-reactive protein and Fibrinogen. World J Preventive Med 2013; 1(3):43-49.
31. Glurich I, Grossi S, Albini B, Ho A, Shah R, Zeid M, et al. Systemic inflammation in cardiovascular and periodontal disease: Comparative study. Clin Diagn Lab Immunol 2002; 9: 425-32.
32. Noack B, Genco RJ, Trevisan M. Periodontal infections contribute toelevated systemic C-reactive level. J Periodontol 2001; 2: 172-80.
33. Kamil W, Habashneh W, Khader Y, Bayati L, Taani D. Effects of nonsurgical periodontal therapy on C-reactive protein and serum lipids in Jordanian adults with advanced periodontitis. J Peridontol Research 2011; 45: 616-21.
34.

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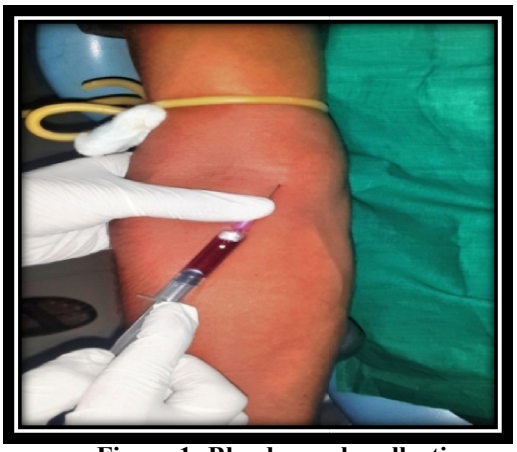

Figure.1: Blood sample collection

Figure 2: Centrifugation of blood to separate the serum from blood.

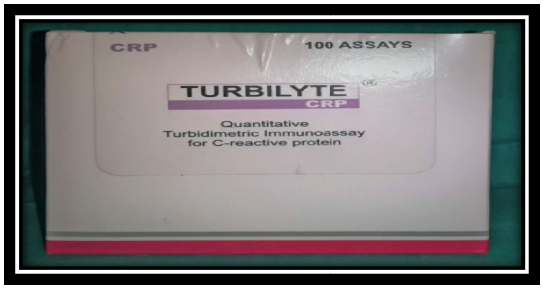

Figure 3: Turbilyte –CRP Kit with Reagent 1, Reagent 2 & Calibrator

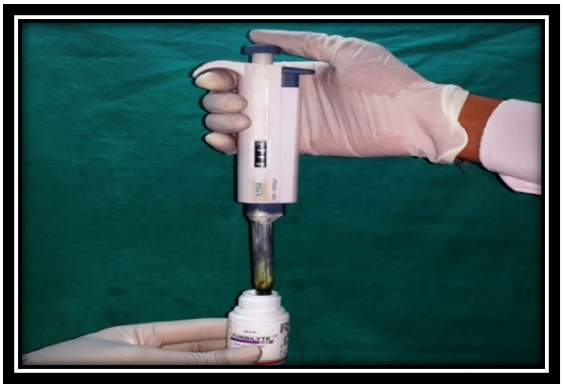

Figure.4: Collecting R1 in Micropipette

Figure 5: Collecting R2 in Micropipette

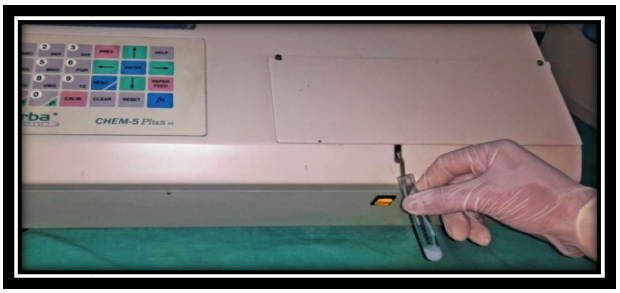

Figure 6: Test Sample (R1+R2+Serum) in Semi Auto analyzer

Table 1: Intergroup comparison of various clinical parameters at Baseline for Group II (GCP) and Group III (GAP) with control group

Study Parameters	Group I (C)	Group II (GCP)	Group III (GAP)	P -value	POST-HOC TEST
	Mean ±SD	Mean ±SD	Mean ±SD		
Plaque Index PI†	0.80±0.16	1.49±0.20	1.28±.09	P<0.001	GCP>GAP>C
Calculus Index CI‡	0.65±0.22	1.61±0.37	1.51±0.24	P<0.001	GCP>GAP>C
Gingival Index GI†	0.69±0.24	1.66±0.15	1.48±0.22	P<0.001	GCP>GAP>C
Bleeding index BI†	0.45±0.08	0.82±0.13	0.72±0.13	P<0.001	GCP>GAP>C
Pocket depth PD†	1.74±0.14	6.85±0.34	6.82±0.36	P<0.001	GAP, GCP>C
Clinical Attachment Level AL†	2.51±0.25	7.27±0.47	7.19±0.23	P<0.001	GAP, GCP>C
C- reactive protein CRP†	0.34±0.09	0.71±0.24	1,19±0.64	P<0.001	GAP>GCP>C

P-value of <0.05 was considered statistically significant.
† ANOVA with post-hoc Games Howell test
‡ANOVA with post-hoc Turkey's test

Table 2: Comparison of various clinical parameters for GCP & GAP Group at Baseline with control group

Baseline			Plaque index PI	Calculus index CI	Gingival index GI	Bleeding index BI	Probing depth PD	Clinical attachment level (CAL)
GAP	CRP	Pearson Correlation	.064	-.325	.127	.152	.361	.255
		p-value	.762	.113	.545	.468	.077	.219
GCP	CRP	Pearson Correlation	.175	.211	.291	.050	.017	.174
		p-value	.402	.310	.159	.814	.934	.407
Controls	CRP	Pearson Correlation	.085	.162	.168	-.086	.220	.058
		p-value	.685	.440	.422	.684	.290	.782

- Exactly –1. A perfect negative linear relationship
- –0.70. A strong negative linear relationship
- –0.50. A moderate negative relationship
- –0.30. A weak negative linear relationship
- 0. No linear relationship
- +0.30. A weak positive linear relationship
- +0.50. A moderate positive relationship
- +0.70. A strong positive linear relationship

Exactly+1. A perfect positive linear relationship