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Comparative evaluation of high sensitivity C - reactive protein (HS-CRP) in patients with healthy gingiva and chronic generalized periodontitis (CGP) without coronary artery disease (CAD)

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Abstract

Objective: The aim of the study was to compare and evaluate levels of hs-CRP in the serum samples of patients with healthy gingiva and CGP patients without CAD.

Material And Method: A total of 40 systemically healthy patients were selected and divided into 2 groups- Patients with healthy gingiva (Group A) and with Chronic Generalized Periodontitis (Group B). The periodontal status was assessed and peripheral blood was collected and hs-CRP levels were estimated in the serum samples.

Result: hs-CRP levels were compared and evaluated in patients with CGP and healthy gingiva. The mean hs-CRP levels in Group A recorded was 0.30 ± 0.208 and Group B was 2.25 ± 0.788. Pearson correlation was used to analyze the relationship between hs-CRP and probing pocket depth. When correlation between PD and hsCRP was evaluated then a statistically highly significant correlation was found ($r = 0.976$, $p > 0.01$).

Conclusion: Patients with chronic generalized periodontitis(CGP) revealed higher levels of hs-CRP than in healthy gingiva patients and this strong association between CGP and hs-CRP revealed the susceptibility to cardiac diseases such as Myocardial infarction and Coronary Artery diseases.

Keywords: Acute-phase reaction, chronic generalized periodontitis, coronary artery disease, high sensitivity C - reactive protein.

Introduction

Periodontitis is the inflammatory disease of the supporting tissues of the teeth which results in pocket formation and loss of alveolar bone.[1] This condition is caused by a chronic, mixed infection of Gram-negative bacteria, such as *P. gingivalis*, *P. intermedia*, *T. forsythia* and *A. actinomycetemcomitans* and Gram-positive bacteria such as *Peptostreptococcus* and *S. intermedius*.[2]

Patients suffering from severe periodontitis have increased local production of inflammatory cytokines (IL-7β, TNF and IL-6) and moderate systemic inflammatory response (defined by raised concentration of C-reactive protein, fibrinogen and moderate leukocytosis).[3] C-reactive proteins (CRP) is a strong, type-I acute phase protein, which is synthesized by hepatocytes and other cell types, including monocytes, endothelial cells, fibroblast and adipocytes.[4]

Increased levels of acute-phase proteins have been noted with gingival inflammation and periodontitis, showing the disease condition.[5] The concentration of acute phase proteins is altered by at least 25% in response to infection.[6]

C-reactive protein (CRP) was the first protein to be discovered by **Tillett and Francis(1930)**.[7] It was named so because it was discovered as a substance in the serum of patients with acute inflammation that reacted with the C-(capsular) polysaccharide of Pnuemococcus.

CRP is a member of pentraxin family of proteins. They are ligands for leukocyte Fcγ(fragment crystallizable-gamma) receptors. Hepatocytes help in production of CRP which is an acute phase protein as increases in plasma concentration during infection and inflammation and is a golden marker of inflammation.[8]

Plasma levels of CRP may rise rapidly as much as 1000-fold after an acute inflammatory stimulus, largely reflecting increased synthesis by hepatocytes. C-reactive protein is a phylogenetically highly conserved plasma protein produced in response to inflammation.[9] It is produced in response to many forms of injury other than periodontitis, such as other infections, trauma and hypoxia, and it is regulated by cytokines such as interleukin-6 (IL-6), IL-1β and tumor necrosis factor-α. CRP levels have an association with smoking, obesity, triglycerides, diabetes, and periodontal disease.[10] It is proposed that changes in cellular and molecular components of peripheral blood can be found in patients with periodontitis because of inflammatory changes of the periodontal tissues.[11]

CRP levels can be measured using immuno-turbidimetric or immune electrophoretic assays or latex slide agglutination method. The high-sensitivity CRP assay uses labeled monoclonal or polyclonal anti-CRP antibodies in an enzyme-linked immunosorbent assay (ELISA) or an immunofluorescent assay. It can also be measured using Chemiluminescent Imnnunoassay (CLIA). It can determine CRP levels in serum as low as 0.15mg/L.[12]

Thus, the purpose of this study was to compare and evaluate the systemic levels of CRP in the serum sample of the patients with healthy gingiva and chronic periodontitis and compare them with the periodontal clinical parameters.

Materials And Method

In this study, forty patients (25 males, 15 females) of age group 30-55 years with good systemic health diagnosed with healthy gingiva and chronic periodontitis were selected from the out-patient department of College of Dental Sciences and Research Centre, Manipur, Ahmedabad, Gujarat, India. The study was approved by ethical committee of College of Dental Sciences and Research Centre before the commencement of the study.

Forty patients were examined under the inclusion and exclusion criteria and they were selected for the study. Both males and females of age group 30-55 years were selected for the study. All the patients were informed about the purpose of the study prior to participation. A verbal and written consent form, duly signed by each patient was obtained.

Selection Criteria

Inclusion Criteria

- Patient with good systemically healthy status.
- Patients having a minimum of 20 natural teeth.
- Patients diagnosed with chronic generalized periodontitis with at least 5 teeth having probing depth (PD) ≥ 5 mm.
- Patients who does not require any dental treatment which needs antibiotic prophylaxis.
- Patients who did not have any history of a recent myocardial infarction.

Exclusion Criteria

- Person having systemic disease like diabetes, rheumatoid arthritis, cardiovascular disease, gastrointestinal disorder, respiratory disorder etc.
- History and /or presence of any other infections, pregnancy, lactating females and smokers
- Obesity (BMI<25KG/M2)
- Patient who have taken anti-inflammatory and antibiotics during last 6 months
- Patient who have undergone periodontal therapy during last 6 months.

Study Design

Patients so selected were equally divided into two groups:

- **Group A**: Patients with healthy gingiva with no change in color, no signs of redness, edema and no bleeding on probing.
- **Group B**: Patients with chronic generalized periodontitis with marked redness, edema and tendency of spontaneous bleeding on probing, at least 5 teeth having probing depth (PD) ≥ 5 mm.

Methodology

Clinical Examination

The Clinical Examination included:

- Complete medical history
- Clinical parameters
- Blood examination

After the selection of subjects a detailed case history was taken.

Clinical Periodontal Examination

Clinical parameters for periodontal examination were recorded. All the data recording were made by the same examiner.

The parameters recorded were:

- Plaque index (PI) (Loe-1967)
- Gingival index (GI) (Loe and Silness-1963)
- Pocket depth (PD)

Serum Sample Collection

A total of 10ml of venous blood was collected from the patients and transferred to a sterilized test tube and was immediately sent to laboratory for assessment of serum CRP levels by high sensitivity Chemiluminescent Immunoassay (CLIA) kit.

Statistical Methodology And Results

Descriptive statistical analysis was carried out in the present study and the data obtained was suitably tabulated using SPSS version 16.0. All the values were expressed in the form of mean, standard deviation. The parameters were compared between Group A and Group B. The results were obtained using unpaired student 't' test. Karl-Pearson's correlation coefficient test was used to measure the correlation between clinical parameters and hs-CRP levels in Group A and Group B.

Group A: Patients with healthy gingiva

Group B: Patients with chronic periodontitis

p value < 0.05 is significant (S)

p value < 0.01 is highly significant (HS)

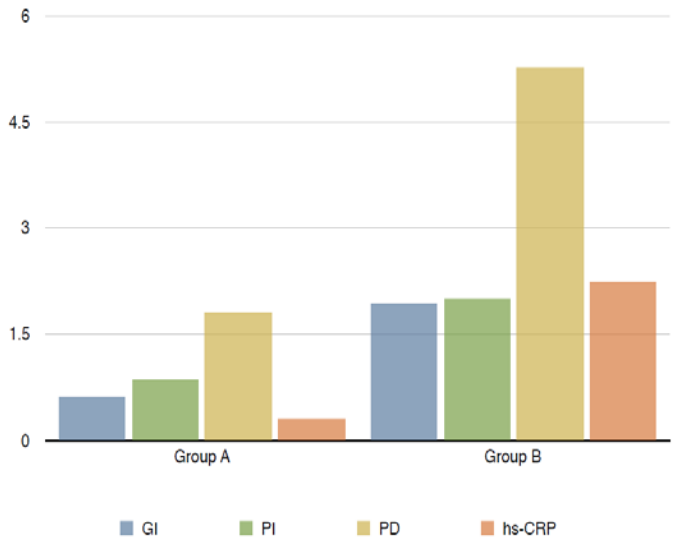
Table 1: Demographic Study

STUDY DESIGN	TOTAL NO. OF CASES	SEX		AGE RANGE (IN YEARS)	TOTAL NO. OF GROUPS	
CROSS SECTIONAL STUDY	40	MALE	FEMALE	30 - 55	GROUP A	GROUP B
		25	15		20	20

Table 2: Mean Values of Clinical Parameters and hs-CRP in Group A and Group B

	Group A	Group B	P Value
GI	0.613 ± 0.233	1.928± 0.292	S
PI	0.863 ± 0.175	2.0± 0.334	S
PD	1.797 ± 0.211	5.257 ± 0.655	S
hs-CRP	0.30 ± 0.208	2.25 ± 0.788	S

Graph 1: Mean Values of Clinical Parameters and hs-CRP in Group A and Group B



GI- Gingival Index PI- Plaque Index

PD- Pocket Depth hs-CRP- High Sensitivity C-Reactive Protein

Mean values for hs-CRP in patients with periodontal disease were increased compared to control subjects (2.25 ± 0.788 mg/l, compared to 0.30 ± 0.208 mg/l). A significant difference was observed between Group A and Group B for all the parameters (p value < 0.05).

Table 3: Pearsons's Correlation Coefficient among hs-CRP levels with Different Clinical Parameters in Group A and Group B.

	GROUP A		GROUP B	
	Pearson's Coefficient correlation 'r'	p Value	Pearson's Coefficient correlation 'r'	p Value
GI	0.974	HS	0.870	HS
PI	0.972	HS	0.946	HS
PD	0.976	HS	0.857	HS

Correlation is highly significant (p value < 0.01)

The variation in hs-CRP values was correlated with variation changes in the different clinical parameters, which was determined using Karl-Pearson's coefficient correlation test. A positive correlation between the clinical parameters and CRP levels were observed in Group A and Group B.

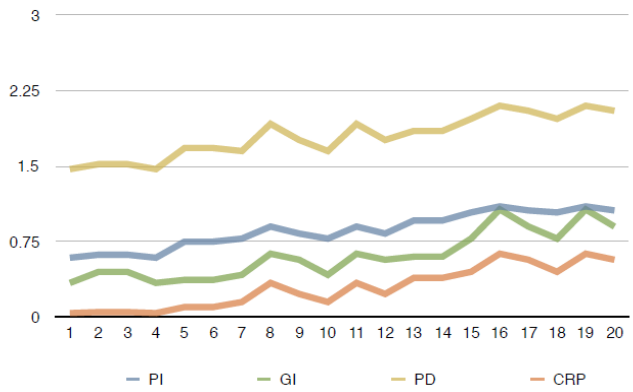
Graph 2: Correlation among Clinical Parameters and the hs-CRP levels in Group A

When correlation between GI and hs-CRP was evaluated then a statistically highly significant correlation was found (r = 0.974, p > 0.01).

When correlation between PI and hs-CRP was evaluated then a statistically highly

significant correlation was found (r = 0.972, p > 0.01).

When correlation between PD and hs-CRP was evaluated then a statistically highly significant correlation was found (r = 0.976, p > 0.01).



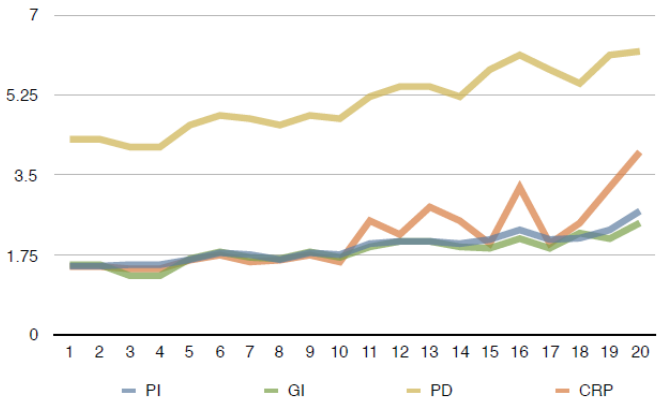
Graph 3: Correlation among clinical parameters and the hs-CRP levels in Group B

When correlation between GI and hs-CRP was evaluated then a statistically highly significant correlation was found (r = 0.870, p > 0.01).

When correlation between PI and hs-CRP was evaluated then a statistically highly

significant correlation was found (r = 0.946, p > 0.01).

When correlation between PD and hs-CRP was evaluated then a statistically highly significant correlation was found (r = 0.857, p > 0.01).



Discussion

It has been shown that inflammation plays a role in the pathogenesis of cardiovascular diseases (CVD).[13] Epidemiological studies have also implicated periodontitis as a risk factor for the development of CVD.[14] People with higher hs-CRP values have the high risk of cardiovascular disease and those with lower values have less risk. Patients who have hs-CRP value on the higher side of the normal value have 1.5 to 4 times the risk of having a heart attack as those with hs-CRP values at the low end of the normal value.

The American Heart Association and U.S. Centers for Disease Control and Prevention

have defined risk groups as follows:[15]

- Low risk: less than 1.0 mg/L
- Average risk: 1.0 to 3.0 mg/L
- High risk: above 3.0 mg/L

CRP represents an emerging and dependable marker of the acute-phase response to infection and/or inflammation. As a consequence of its kinetics, it best describes the inflammatory status of the individual.[16]

Periodontal diseases are associated with an increase in CRP levels. This is significant because CRP is a widely accepted measure of the levels of systemic inflammation and increase in CRP levels are associated with an increased risk of atherosclerosis.[17] Periodontal disease also may lead to transient increase in circulating levels of IL-1β, TNF-α and prostaglandin E2. This may be the first step in the contribution of periodontal diseases to systemic inflammation.[18]

CRP is a trace protein in healthy patients, the median value being 0.8mg/l, with a interquartile range of 0.3 to 1.7 mg/l.[12] CRP is normally present in low quantities in human serum, but may increase 100 to 1000-fold within 72 hours of tissue injury.[19] CRP concentrations closely follow the course of the acute-phase response to inflammation or tissue necrosis, and so can theoretically provide a valuable measurement for many disease processes.[20]

Forty patients (25 male and 15 female) of age group 30-55 years with good systemic health diagnosed with healthy gingiva and chronic periodontitis were selected **(Table 1)**. Clinical parameters like Gingival Index (GI), Plaque Index (PI) and probing depth (PD) were used for the assessment of the periodontal conditions. These indices are the methods for quantifying the amount and severity of diseases or conditions in individuals.

In the present study, CRP levels in patients with periodontal disease were compared to patients with healthy gingiva and for that the high sensitivity CRP Chemiluminescent Immunoassay (CLIA) kit was used to determine the values of CRP with a sensitivity range of 0.01 to 10 mg/ l. This technique provides a sensitive and economical way to quantitatively measure CRP in serum samples. Chemiluminescent Immunoassay does not require long incubations and the addition of stopping reagents, as it is in the conventional colorimetric assays such as Enzyme-linked ImmunoSorbent Assays (ELISA). The kits provide a broader, dynamic range, high sensitivity and a faster protocol than the conventional ELISA method.[21]

In the present study, the mean Gingival Index (GI) score in Group A was 0.613 ± 0.233 and Group B was 1.928 ± 0.292 **(Table 2)**. Gingival Index has shown a positive correlation with the levels of hs-CRP in patients with chronic periodontitis group suggesting that increase in the microbial colonization leads to the increased levels of CRP in serum. ($r = 0.870$, $p > 0.01$) **(Table 3)**. A significant difference was observed between Group A and Group B for Gingival Index ($p < 0.05$) **(Table 2)**.

The results obtained were in accordance with the study done by **Mahdieh et al. (2013)**.[22] A significant difference was obtained between the periodontitis patients and healthy subjects on comparing GI and CRP levels.

The mean Plaque Index (PI) score in Group A was 0.863 ± 0.175 and in Group B was 2.0 ± 0.334. When correlation between Plaque Index and hs-CRP was evaluated then a statistically highly significant correlation was found ($r = 0.946$, $p > 0.01$) in patients with chronic periodontitis **(Table 3)**. A significant difference was observed between Group A and Group B for Plaque Index ($p < 0.05$) **(Table 2)**. This proves that the severity and the extent of the infection causes the rise in CRP levels.

The results obtained were in accordance with the study done by **Ana Pejcic et al. (2011)**[23] where he found all the periodontal parameters (PI, GI, BOP and PPD) were significantly correlated with CRP levels in periodontitis patients. ($p < 0.001$).

Along with this, CRP levels increased with the increase in the probing depth (PD) in all the patients with chronic periodontitis. The mean probing depth in Group A was 1.797 ± 0.211 and in Group B was 5.257 ± 0.655 **(Table 2)**. Statistically highly significant correlation was found between probing depth and hs-CRP in Group B ($r = 0.857$, $p > 0.01$) **(Table 3)**.

This is in the accordance with the studies done by **Ide M et al. (2003)**[24] and **D' Auito et al. (2004)**[25] where a positive correlation between CRP and periodontitis patients with probing pocket depth of ≥ 4 mm was found.

In the present study, CRP levels were compared and evaluated in the patients with chronic periodontitis and patients with healthy gingiva. The mean hs-CRP levels in Group A recorded was 0.30 ± 0.208 and in Group B was 2.25 ± 0.788. A significantly elevated hs-CRP level was found in Group B as compared to Group A ($p < 0.05$) **(Table 2)**. Thus it was concluded that the patients with chronic periodontitis demonstrated a mean hs-CRP levels higher than the patients with healthy gingiva. This indicates that there is a significant association between periodontitis and serum CRP concentrations.

The results obtained were in accordance with the study done by **Bansal et al. (2014)**.[26] They compared the CRP levels in the serum sample of the patients with healthy gingiva (Group A), gingivitis (Group B) and chronic periodontitis (Group C). The mean hs-CRP level in Group A recorded was 0.437 ± 0.216, Group B was 0.771 ± 0.384 and Group C was 2.285 ± 0.381. A significantly elevated hs-CRP level was found in Group C as compared with Group B and A ($P < 0.05$). Thus, it was concluded that the patients with chronic periodontitis demonstrated a mean hs-CRP levels higher than the patients with gingivitis and with healthy gingiva.

Thus, in this study, elevated levels of CRP was observed in the patients with periodontitis as compared to patients with healthy gingiva. Also, patients with the periodontitis infection with elevated level of CRP are at a higher risk of future CHD.

CRP is said to be the strongest and most significant predictor of the risk of future cardiovascular events.[2]

Conclusion

The present study was carried out to compare and evaluate the serum CRP levels of the patients with healthy gingiva and chronic periodontitis and compare them with the periodontal clinical parameters. Through this study, it could be concluded that:

1. There was significant increase in the levels of hs-CRP in patients with periodontitis then the patients with healthy gingiva
2. There was a positive correlation between the clinical parameters and the hs-CRP levels in the periodontitis patients.
3. Inflammatory condition of the gingiva results in elevated levels of CRP.
4. Also, the measurement of serum CRP can be used as a reliable test for the detection and screening of periodontal disease in systemically healthy people.

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