Comparative Evaluation Of C-Reactive Protein Levels And Leucocyte Counts In Patients With Sickle Cell Anemia, Chronic Generalized Periodontitis And Sickle Cell Anemia With Chronic Periodontitis: A Clinico-Biochemical Study

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Abstracts: Aim: The aim of this study is to compare the CRP levels and leucocyte counts in patients with Sickle cell anemia, Sickle cell anemia with chronic periodontitis, and chronic periodontitis. Material andMethods: A total of 90 subjects with an age range of 30-50 years having sickle cell anemia and chronic periodontitis with probing depth ≥5mm and clinical attachment level ≥ 5mm were included and three groups: Group I- Sickle cell anemia; Group II- Sickle Cell anemia with chronic periodontitis and Group III: Chronic severe periodontitis. Blood samples for CRP and leucocytes counts estimation were collected. All participants were subjected to quantitative CRP analysis. Results: Mean CRP levels and leucocyte counts were significantly greater in group II as compared to group I and group III. Conclusion**:** The present study indicates a positive correlation in CRP levels and leucocyte counts in patients with Sickle cell anemia, chronic periodontitis and both. [Sachin M NJIRM 2016; 7(4):22-27]

Key Words: Chronic generalized periodontitis (CGP), C‑reactive protein (CRP), leucocyte counts, Sickle cell anemia (SCA)

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Introduction: Growing interest in association between periodontal disease and systemic inflammation has led to convergence in oral and medical care. It has been widely accepted that periodontal infections are leading culprits for cardiovascular disease (CVD). This association could partly be explained by C‑ reactive protein (CRP) and leucocyte, which are systemic markers of inflammation.It is receiving the most attention as it best describes the inflammatory status of an individual due of its kinetics.[1] Also, C-reactive protein (CRP) and leucocyte counts are the biomarkers of sickle cell disease (SCD).[2]

CRP is an acute‑ phase reactant produced by the liver, [3] which is nonspecific and produced in response to various stimuli. CRP possesses the ability to reveal inflammation at an early stage as it rises in serum within 48 h.[4] 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, which directly reflects the intensity of the pathological process stimulating CRP production.[5]

This property is useful for early detection of patients who are at risk for inflammatory disease. Moreover, it can upregulate proinflammatory mediator production.6 In SCD patients, there is an increase in CRP levels due to vaso-occlusion.[7] SCD is characterized by abnormally elevated total white cell count,secondary to hyposplenism and inflammation. Unusually high white cell count in SCD may be prognostic indicator of clinical severity.[8] A number of studies have demonstrated an association between periodontal disease and the risk of CVD.[9,10] CRP and leucocyte possess the ability to predict future cardiovascular events in apparently healthy individuals.

Periodontitis is a local inflammatory process mediating destruction of periodontal tissues triggered by bacterial insult. It is characterized by systemic inflammatory host responses, which, may contribute, in the part, of the recently reported higher risk of cardiovascular disease (CVD) among patients with periodontitis.[11] C-reactive protein (CRP) and leucocyte counts are an acute-phase reactants/ and have been proved to be a significant predictor of future cardiovascular events and are said to be biomarkers of SCD. Thus, the aim of the present study was to determine and compare the CRP levels and leucocyte counts in patients with Sickle cell anemia, chronic periodontitis and sickle cell anemia with chronic periodontitis. The study was carried out according to Ethical Standards of the 1975 Helsinki Declaration and was approved by Institutional review board, MCDRC, Durg.

Methods: Following complete medical and dental examination, 90 individuals were selected for the study from Department of Periodontics, Maitri College of Dentistry and Research Centre, Durg, CG, India.
A total of 90 subjects with an age range of 30-50 years having sickle cell anemia, chronic generalized

periodontitis with probing depth ≥5mm and clinical attachment level (CAL) ≥ 5mm were divided into three groups:

- Group I, sickle cell anemic patients (SCA);
- Group II, sickle cell anemia with chronic generalized periodontitis (SCAP) patients and
- Group III, chronic generalized severe periodontitis (CGP) patients. Informed consent was obtained from each individual.

Inclusion criteria:

Subjects were placed into three groups according to the following definitions:

- •Group I: Sickle cell anemic (SCA), non-periodontitis (NP) group - clinically healthy periodontal status, with no evidence of attachment loss.
- •Group II: Sickle cell anemic with chronic periodontitis (SCAP), probing depth (PD) of ≥5 mm and/or clinical attachment loss (CAL) ≥ 5mm.
- •Group III: Chronic severe periodontitis (CGP), probing depth (PD) of ≥5 mm and/or clinical attachment loss (CAL) ≥ 5mm (Severe Periodontitis).

Exclusion criteria:

- Current smokers;
- Pregnant and lactating women;
- Patient undergone oral prophylaxis for the past 6 months, and
- Patient under antibiotics or any drug which can affect gingival health in the preceding 6 months.

Periodontal assessment

Periodontal disease status was evaluated by measuring the probing depth (PD), CAL, using the same periodontal probe (UNC- 15 probes Hu- Friedy's, USA).

Sample collection: Venous blood was withdrawn from the participants selected for the study. The subjects were informed, and 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. After cleansing the puncture site with 10% isopropanol solution, blood was withdrawn using a syringe with 24 gauge needles. Tourniquet was released as the blood flow began. After drawing 4 mL of blood, sterile cotton ball was placed on the puncture site and needle was withdrawn and patient was asked to loosen the fist. The subjects were instructed to apply mild finger pressure on the site for few minutes to avoid oozing out of blood.

C- reactive protein determination

The CRP serum level of each patient was quantified using a commercial high-sensitivity radial immunodiffusion kit for human CRP. The test was performed according to the instructions of the manufacturer. Samples concentration (mg/L) corresponding to each ring diameter were read from RID Reference Table after complete development of the rings. A calibrator was run on each plate to validate test performance. The lower and upper detection limits of this kit are 0.18mg/L and 8.0mg/L, respectively. A kit elevated CRP levels was used for assaying samples with CRP concentration >8.0mg/L.

Leucocyte Counts

2ml of total collected blood sample was placed in the test tube containing anti-coagulant (EDTA). The sample is then transported to a laboratory and was processed using a Pasteur pipette for immediate processing by an automated counter.

Statistical analysis

Data were presented as mean, standard deviation and 95% confidence interval of the mean difference. Comparison of three groups with respect to CRP and leucocyte counts was done using one- way analysis of variance (ANOVA).

Result: Table 1 and figure 1 provide the mean CRP levels in three groups. Leucocyte counts of three groups were summarized in table 2 and figure 2. The mean CRP levels of the group I, II and III are 8.73, 10.72 and 8.16 respectively. CRP values and leucocyte counts of the three patient groups were significantly different from each other, with CRP levels and leucocyte counts in the group II greater than those in the group I, which were in turn greater than those in the group III subjects in figure 1and 2.

Furthermore, comparison of three groups with respect to CRP values and leucocyte by one- way ANOVA showed statistical significance, between group I and group II; group II and group III, but no statistical significant difference was observed between group I and III, as shown in table 1a and 2a. Again, they were significantly correlated, and the increasing CRP and leucocyte counts, thus, indicating positive correlation.

Table: 1 Study variables in comparison between Serum CRP levels

C-REACTIVE PROTEIN (CRP) in mg/L	Mean	SD	F ratio (ANOVA)	P value
Group I	8.73	2.49	5.56	0.002 HS
Group II	10.72	2.09		
Group III	8.16	2.98		

Values are presented as mean, SD: standard deviation, group I: SCD, group II: SCDP, group III: CGP

Figure 1: Graphic representation of CRP levels in groups

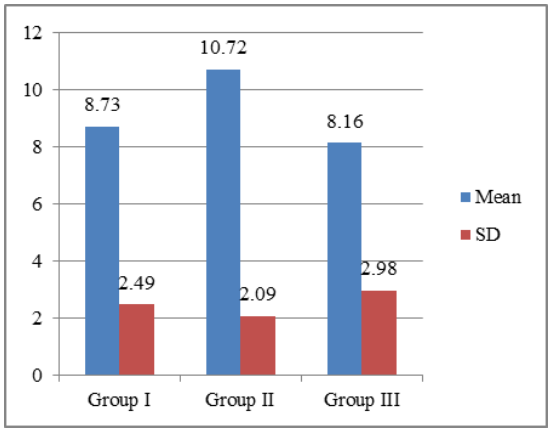

Table 1a: Comparison between groups with respect to CRP levels

C-REACTIVE PROTEIN	Group II	Group III
Group I	P<0.01 HS	NS
Group II		P<0.01 HS

HS: Highly Significant; NS: Not Significant

Table 2: Leucocyte counts in three groups:

LEUCOCYTE COUNTS	Mean	SD	F ratio (ANOVA)
Group I	11190.33	1301.29	6.33
Group II	12626.67	2975.06	
Group III	10103.33	2150.78	

Figure 2: Graphic presentation of the leucocyte counts in groups

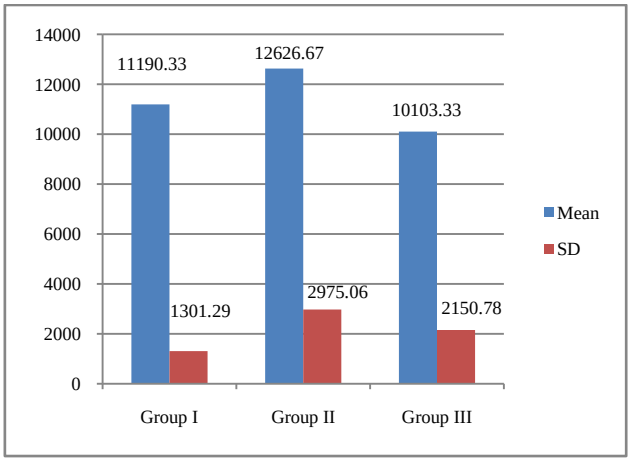

Table 2a: Comparison of three groups in respect to three groups

LEUCOCYTE COUNT	Group II	Group III
Group I	P<0.05 HS	NS
Group II		P<0.01 HS

Discussion: Main finding of the study was that CRP levels and leucocyte counts were higher in all the groups but was highest in group II when compared with other two groups, demonstrating that sickle cell anemia and chronic periodontitis can have an impact on systemic markers of inflammation.

CRP is a direct and quantitative measure of the acute phase reaction. The amount of CRP produced by the body varies from person to person, and this is affected by an individual's genetic makeup (accounting for almost half of the variation in CRP levels between different people) and lifestyle. After stimulation of the hepatocytes by cytokines, levels of CRP in the blood start to increase within 6 h. These concentrations can increase up to 1000-fold or even more. Smoking and obesity are positively correlated with CRP levels, whereas weight loss and cessation of smoking decrease CRP values.

CRP levels correlated positively with the severity of inflammation as it is biologically plausible that inflammatory cytokines interleukin‑ 6 (IL‑ 6), IL‑ 1, and tumour necrosis factor‑ alpha in response to periodontal infection present the capacity to stimulate hepatocyte to produce CRP. Circulating CRP levels are markers of systemic inflammation and are associated with periodontal disease.[12] Also of significance are the results of prospective longitudinal trials which indicate that this marker appears to be a useful predictor for future cardiovascular events in a variety of population.[13]

Leukocytes contribute to SCD by adhering to blood vessel walls and obstructing the lumen, aggregating with other blood cells with more effective blockage of the lumen, stimulating the vascular endothelium to increase its expression of ligands for adhesion molecules on blood cells, and causing tissue damage and inflammatory reaction which predispose to vaso-occlusion. Patients with impaired ability of leukocytes to kill microbes are more prone to infections; which precipitate sickle cell crisis.[14]

Okocha et al studied C-reactive protein levels in Nigerian Sickle cell patients and found significant elevated levels of CRP.[15] Similar results were found by Walter et al,[16] Zacho et al,[17] and Olaniyi J et al. [18] Charache hypothesized that SCD is characterized by abnormally increased leucocyte counts. In the light of available evidence, white blood cells most probably contribute to the pathogenesis of the vaso-occlusive events that characterize sickle cell disease. [19]

Several cross-sectional and longitudinal epidemiologic studies are consistent in reporting that periodontitis patients are at a higher risk for cardiovascular diseases including coronary artery disease and stroke. The association is hypothesized to be linked to direct effects of periodontal pathogens or indirect host-mediated effects triggered by infection.[20] Systemic inflammatory host responses in periodontitis are suggested to be one of the possible underlying mechanisms of this relationship.[21] Several recent studies have emphasized that even moderately elevated CRP serum levels are predictors of increased risk of cardiovascular disease among apparently healthy individuals. Chronic bacterial infections such as periodontitis are one of the established risk factors for moderate elevated CRP level.[22-27]

This study shows an association between elevated serum CRP level and leucocyte counts in patients with sickle cell anemia and chronic periodontitis. For better understanding, the nature of association, long‑ term longitudinal studies are needed. Limited sample size, the role of genetics, oral health behaviours, nutrition, stress levels, which have been shown to affect the prevalence of periodontitis, and various blood and urine biomarkers have been identified in SCD were not considered in our study. From our study, we can conclude that SCD and periodontitis are related with the severity of inflammation, so it is empirical to treat the SCD and periodontal inflammation but recommending periodontal therapy solely for the purpose of preventing CVD is not supported by current evidence of literature. Recommendations by American Heart Association states that patients should be educated about oral health as no one is systemically healthy without possessing good oral health.

Conclusion: In the present study, there was positive correlation in CRP levels and leucocyte counts in patients with Sickle cell anemia, chronic periodontitis and both. As the goal of modern health care continues to shift from an attitude of treatment to one of prevention, more longitudinal studies with larger sample size and specific investigations are required to validate our findings.

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