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Comparative Estimation of C-Reactive Protein in Gingival Crevicular Fluid of Patients with Periodontitis and Myocardial Infarction in Remission

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Abstract: ***Background**: C-reactive protein (CRP), a sensitive marker of systemic inflammation, has been implicated in the pathogenesis of both periodontitis and cardiovascular diseases. Gingival crevicular fluid (GCF) reflects local inflammatory activity and may serve as a non-invasive medium to explore the periodontal-cardiovascular interface. **Aim**: To estimate and compare C-Reactive Protein levels in gingival crevicular fluid of patients with chronic periodontitis, and those with chronic periodontitis and a history of myocardial infarction (MI) in remission. **Materials and Methods**: This cross-sectional study included 60 participants divided into three groups (n = 20 each): Group I (healthy controls), Group II (chronic periodontitis), and Group III (chronic periodontitis with MI in remission). GCF was collected using calibrated microcapillary pipettes, and CRP concentrations were quantified using a high-sensitivity ELISA. Intergroup comparisons were performed using ANOVA and post hoc analysis. **Results**: Mean GCF CRP levels were significantly higher in Group III compared to Groups II and I ($p < 0.001$), demonstrating a graded increase corresponding to systemic disease burden. **Conclusion**: Elevated GCF CRP levels in patients with periodontitis and MI in remission suggest an enhanced local inflammatory response, reinforcing the bidirectional link between periodontal and cardiovascular health.*

Keywords: C-reactive protein, gingival crevicular fluid, periodontitis, myocardial infarction, inflammation, biomarkers

1. Introduction

Periodontitis is a multifactorial, chronic inflammatory disease characterized by progressive destruction of the periodontal ligament and alveolar bone [1]. The dysregulated host immune response to a pathogenic subgingival biofilm contributes to both local tissue destruction and systemic inflammatory dissemination. In recent decades, considerable attention has been directed toward elucidating the bidirectional relationship between periodontal disease and systemic conditions, particularly atherosclerotic cardiovascular disease (ASCVD) [1, 2].

C-reactive protein (CRP), an acute-phase reactant synthesized by hepatocytes in response to interleukin-6 and other pro-inflammatory cytokines, has emerged as a robust biomarker for systemic inflammation [2]. Elevated serum CRP levels have been strongly associated with adverse cardiovascular events, including myocardial infarction (MI), and are predictive of both disease severity and recurrence [3]. Concomitantly, periodontitis has been shown to elevate systemic CRP levels, suggesting a shared inflammatory axis between periodontal and cardiovascular pathology [2, 3].

While the majority of investigations have focused on circulating CRP levels, the presence of CRP in gingival crevicular fluid (GCF) represents a more localized, site-specific indicator of periodontal inflammation. GCF, as a transudate of the periodontal microenvironment, mirrors the host immune response at the tissue level [3]. Importantly, patients with a history of MI may present with persistent low-grade systemic inflammation, even during clinical remission, potentially influencing local periodontal inflammatory markers such as CRP [4].

Despite growing recognition of the interplay between periodontal disease and cardiovascular health, there remains a paucity of data evaluating local CRP expression in periodontal tissues of patients with previous MI [4,5]. Understanding the CRP profile in GCF may provide novel insights into localized inflammatory burden in this patient population and may help stratify cardiovascular risk from a periodontal perspective [5].

Accordingly, the present cross-sectional study was designed to estimate and compare CRP levels in GCF among systemically and periodontally healthy individuals, patients with chronic periodontitis, and patients with chronic periodontitis with a prior history of MI in remission [5,6]. By assessing CRP levels at the periodontal site, this study aims to clarify the extent to which systemic cardiovascular events modulate local periodontal inflammation, contributing to the growing evidence supporting a periodontal–cardiovascular inflammatory continuum [6].

2. Materials and Methodology

2.1 Study design

This cross-sectional observational study was conducted in the Department of Periodontology, Faculty of Dental Sciences and Central Research Laboratory, Ramaiah University of Applied Sciences, Bangalore, after obtaining approval from the Institutional Ethics Committee. All procedures adhered to the Declaration of Helsinki (2013),

and written informed consent was obtained from all participants.

2.2 Study Population

Sixty participants aged 35–65 years were recruited from the outpatient clinic. Subjects were divided equally into three groups (n = 20 each):

- **Group I (Healthy Controls):** Clinically periodontally healthy individuals with no systemic disease, exhibiting probing pocket depth (PPD) ≤3 mm, no clinical attachment loss (CAL), and absence of bleeding on probing [7].
- **Group II (Chronic Periodontitis):** Systemically healthy individuals diagnosed with Stage II or III, Grade B periodontitis, based on the 2017 AAP/EFP classification, with ≥30% of sites showing CAL ≥3 mm and PPD ≥5 mm [7].
- **Group III (Chronic Periodontitis + MI Remission):** Individuals with chronic periodontitis (criteria as in Group II) and a medically documented history of myocardial infarction, in clinical remission for ≥6 months, without recent cardiovascular events or interventions. Cardiac remission status in Group III was confirmed through recent medical records. A single calibrated examiner (kappa > 0.85) conducted all periodontal assessments [7].

2.3 Inclusion and Exclusion criteria

Inclusion Criteria: Age Groups: 30 to 70 years

- No systemic diseases and not to be on any medication except for patients with Myocardial infarction (MI) [6].
- Patients with no bleeding on probing (BOP<10%), probing depth (PD) ≤3 mm, no clinical attachment loss (CAL), no radiographic evidence of marginal bone loss (MBL) [6].
- Presence of at least 15 teeth [6,7]
- Stage III periodontitis patients with radiographic MBL extending apically to the middle third of the root [6,7].
- Probing pocket depth (PPD) was measured using William's periodontal probe, at six sites for each tooth and was ≥6mm [6,7]
- MI diagnosis with or without ST-segment elevation of >2 mm in the electrocardiography, low-density lipoprotein and high-density lipoprotein concentration [7,8].

Exclusion Criteria:

- On contraceptive drugs in the six months before the study [7]
- On anti-inflammatory, immunosuppressants or antibiotics or drugs in the six months before the study [6,7,8]
- Pregnancy or lactation [6,7,8]
- Periodontal treatment in the past six months prior to the study [6,7,8]

2.4 Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee of Ramaiah University. All procedures involving human participants were conducted in accordance with the ethical standards of the institutional review board and the 2013 revision of the Declaration of Helsinki [9]. Prior to participation, all subjects received detailed verbal and written information regarding the study's purpose, methodology, potential risks, and benefits [9]. Written informed consent was obtained from each participant, confirming voluntary participation and the right to withdraw at any stage without penalty [9].

2.5 Criteria for Determining a Case of Myocardial Infarction in Remission:

Acute myocardial infarction (AMI) was diagnosed based on the patient's clinical presentation and the measurement of serum troponin-I levels exceeding 0.014 ng/mL [8,9]. This diagnostic approach was applied regardless of whether there was associated ST-segment elevation on the electrocardiogram (ECG) [9]. In cases where ST-segment elevation was present, defined as an elevation of more than 2 mm in the precordial leads or more than 1 mm in the limb leads, the infarction was categorized as ST-segment elevation myocardial infarction (STEMI) [8,9]. Conversely, when ST-segment elevation was absent, the infarction was classified as non-ST elevation myocardial infarction (NSTEMI) [9,10]. This classification helps differentiate the two forms of AMI based on the presence or absence of significant ECG changes, which are crucial for determining the appropriate treatment strategy [7,8].

2.6 Laboratory measurements:

The following lipid and apolipoprotein levels were measured: total cholesterol (mg/dL), low-density lipoprotein (LDL) cholesterol (mg/dL), high-density lipoprotein (HDL) cholesterol (mg/dL), apolipoprotein A1 (Apo A1) (mg/dL), apolipoprotein B (Apo B) (mg/dL), and apolipoprotein E

(Apo E) (mg/dL) [7,8,9,10]. In addition, the LDL/HDL ratio was calculated by dividing the HDL cholesterol concentration by the LDL cholesterol concentration. These measurements are critical for evaluating the patient's lipid metabolism and understanding their cardiovascular risk profile, guiding further clinical management [9,10].

2.7 Clinical and Analytical Study Protocol:

Patients were recruited into the study following the acquisition of informed consent. Comprehensive periodontal clinical parameters were recorded, including plaque index (**Silness and Löe**), gingival index (**Löe and Silness**), probing pocket depth (PPD), clinical attachment level (CAL), and bleeding on probing (BOP). For each patient, the sites exhibiting the greatest probing pocket depths were selected for gingival crevicular fluid (GCF) sampling. Prior to sample collection, the designated sites were gently dried with a stream of air and isolated using sterile cotton rolls to prevent contamination from saliva [10, 11, 12].

GCF samples were collected using a microcapillary pipette with standardized internal dimensions [11]. Prior to the collection procedure, the selected sites were carefully prepared to eliminate potential sources of contamination, particularly saliva. Supragingival plaque, if present, was gently removed without traumatizing the soft tissues [10,11]. The sites were then isolated using sterile cotton rolls to

absorb excess saliva, and a gentle stream of air from a dental air syringe was employed to achieve thorough desiccation of the area. This step was critical to maintain the purity of the GCF sample and to prevent dilution or alteration of its biochemical composition [11,12].

Once the sites were adequately isolated and dried, GCF collection was performed by positioning a calibrated microcapillary pipette or tubing at the entrance of the gingival sulcus [12,13]. Care was taken to avoid inserting the pipette into the sulcus, as this could provoke bleeding or induce an inflammatory response, thereby contaminating the sample [11]. The tip of the capillary tubing was gently placed in close proximity to the gingival margin, allowing the fluid to be drawn passively by capillary action without exerting pressure on the tissues [12].

The duration of fluid collection was standardized across all samples to account for individual variations in GCF flow rate. Typically, a time period of 30–60 seconds was allotted for sample acquisition. If the desired volume of GCF was not obtained within the prescribed time frame, the sample was discarded and the procedure was repeated after an adequate waiting period to prevent tissue fatigue [11,12].

In cases where inadvertent contamination with blood occurred, such samples were discarded, and an alternative site was selected for subsequent collection, or, where appropriate, the same site was reattempted following adequate hemostasis and healing [12].

All samples were immediately transferred into pre-labelled sterile Eppendorf tubes and stored at –80°C until further analysis to preserve the integrity of the biomolecules of interest [12]. Particular attention was given to minimizing the time between collection and freezing to prevent proteolytic degradation or alterations in the cytokine or enzyme profiles of the fluid [13].

In the subgroup of patients with periodontitis and a history of myocardial infarction (MI) in remission, detailed clinical histories were meticulously documented, with specific emphasis on the pharmacological regimens administered for MI management [12,13]. Electrocardiographic (ECG) assessments were conducted to evaluate ST-segment elevations, and serum lipid profiles, including low-density lipoprotein (LDL) and high-density lipoprotein (HDL) concentrations, were recorded. The diagnosis of myocardial infarction in these patients was confirmed by a calibrated examiner through a thorough and critical review of medical and diagnostic records [13,14].

Periodontal clinical parameters were recorded at the time of referral for all patients in the MI remission group [13]. The site demonstrating the deepest probing pocket depth was selected for GCF sampling, following the identical standardized procedure of drying, isolation, and atraumatic collection with a microcapillary pipette [14]. The GCF samples were immediately transferred to sterile, airtight plastic vials and stored at –80°C until biochemical evaluation. The CRP levels were assayed using the Human High Sensitive CRP ELISA kit in accordance with the manufacturer's established protocol [14,15].

2.8 CRP Estimation by ELISA

The concentration of C-reactive protein (CRP) in gingival crevicular fluid samples was quantified using a high-sensitivity enzyme-linked immunosorbent assay (ELISA) kit (Manufacturer: Krishgen Biosystems, Lot No: HHSCRP0924), following the manufacturer's protocol. Prior to the assay, all reagents and samples were brought to room temperature (18–25°C) to ensure optimal reaction conditions and eliminate temperature-related variability. Removable 8-well strips were labeled to correspond with standards and samples to avoid cross-contamination and ensure accurate sample tracking [15].

A total of 100 µL of each CRP standard and GCF sample was added to the designated wells [16,17]. The plate was covered and incubated for 2.5 hours at room temperature with gentle agitation to allow antigen-antibody binding. After incubation, the wells were aspirated and washed four times using 300 µL of 1X wash buffer per well to remove unbound material. Residual liquid was eliminated by blotting the inverted plate on absorbent paper [15,16].

Next, 100 µL of the prepared biotinylated anti-CRP detection antibody was added to each well, followed by incubation for 1 hour at room temperature with gentle shaking [17]. After washing as previously described, 100 µL of streptavidin-HRP conjugate was added, and the plate was incubated for 45 minutes [18]. Another washing cycle was performed to remove excess conjugate [16,17].

Subsequently, 100 µL of TMB substrate solution was added, and the plate was incubated in the dark for 30 minutes to allow enzymatic color development [18,19]. The reaction was then terminated by adding 50 µL of stop solution (0.2 M sulfuric acid) to each well, resulting in a color change from blue to yellow [17,18,19]. Absorbance was measured immediately at 450 nm using a microplate reader. The intensity of the color was directly proportional to the CRP concentration in each sample [18,19].

2.8 Statistical Analysis

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses.

Descriptive Statistics: Descriptive analysis of all the explanatory and outcome parameters was done using mean and standard deviation for quantitative variables, frequency and proportions for categorical variables.

Inferential Statistics: One-way ANOVA test followed by Tukey's Post hoc / Kruskal Wallis Test followed by Dunn's post hoc Test was used to compare the levels of mean PI, GI, percentage of Bleeding on probing, PPD, CAL and GCF CRP levels between 3 study groups. Pearson's correlation test / Spearman's correlation test was used to assess the relationship between PI, GI, percentage of Bleeding on probing, PPD, CAL and GCF CRP levels in each group. Multiple linear regression analysis was performed to predict the GCF CRP levels by using clinical parameters in each group The level of significance was set at $P<0.001$.

2.9 Sample size Determination

The sample size for the present study was estimated using GPower software (latest ver. 3.1.9.7; Heinrich-Heine-Universi-ta¨t Du¨sseldorf, Du¨sseldorf, Germany). The sample size estimation was performed at 5% alpha error (α = 0.05), with an effect size of 42% [Based on the findings from the previous literature, study done by Riku Arai et al, 2023 for the relationship between severity of periodontitis and atherosclerotic cardiovascular status in patients with AMI] & the power of the study at 80%, revealed that a minimum of 60 samples will be necessary for the present study. The sample size for present study includes 60 samples [20 samples x 3 groups = 60 samples]

3. Results

A total of 60 adult participants were recruited for the study and systematically categorized into three groups based on inclusion criteria to ensure appropriate representation and comparability. Of the total study population, majority of the participants in all groups were males as compared to females and this difference was not statistically significant. The age of the participants ranged from 30 – 70 years. The mean age of the participants in group 1 was 48.52 ± 8.33, in group 2 was 49.80 ±9.69, in group 3 was 51.87 ±8.73 with a standard deviation of ±5.59 years in each group, indicating a middle-aged cohort with moderate age variability. There was no statistically significant difference in the age group of the participants between the groups (p=0.14) (***Table 1***).

Table 1: Mean Age & Gender distribution among different study groups

Category	Group 1		Group 2		Group 3	
	Mean	SD	Mean	SD	Mean	SD
Mean	48.53	8.33	49.80	9.69	52.87	8.73
Range	32 - 65		35 - 69		40 - 67	
	n	%	n	%	n	%
Males	10	66.7%	12	80.0%	11	73.3%
Females	5	33.3%	3	20.0%	4	26.7%
a. Kruskal Wallis Test & b. Chi Square Test						

A one-way analysis of variance (ANOVA) was conducted to evaluate the differences in gingival crevicular fluid (GCF) CRP levels (ng/ml) among three distinct study groups (Group I, Group II, and Group III). The results are summarized in the table below:

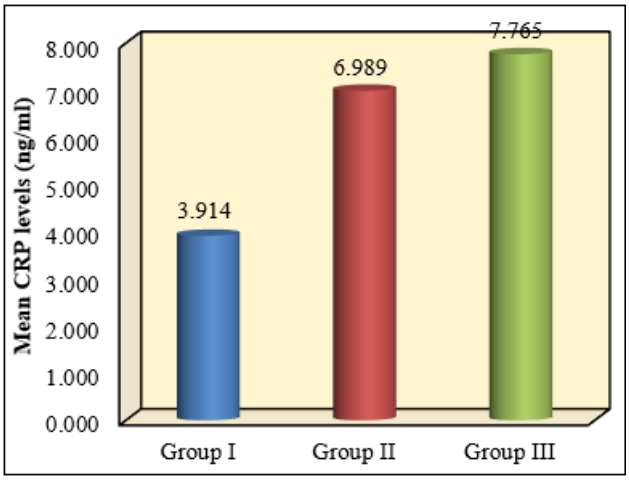
Table 2: Comparison of mean CRP levels (ng/ml) between 3 groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	p-value
Group I	20	3.914	0.674	2.88	5.36	<0.001*
Group II	20	6.989	0.797	5.14	8.46	
Group III	20	7.765	0.683	6.69	9.30	

The mean CRP level in Group I was 3.914 ± 0.674 ng/ml, which was significantly lower than in Group II (6.989 ± 0.797 ng/ml) and Group III (7.765 ± 0.683 ng/ml). The difference in mean CRP levels among the three groups was found to be statistically significant with a p-value < 0.001.

These findings indicate that CRP levels in GCF increase progressively from Group I to Group III, suggesting a possible positive association between systemic inflammatory status and periodontal condition (***Table 2***)

The mean CRP levels differed considerably among the three groups. Group I exhibited the lowest mean concentration at 3.914 ± 0.674 ng/ml, with values ranging between 2.88 and 5.36 ng/ml. In contrast, Group II showed a notable increase, recording a mean level of 6.989 ± 0.797 ng/ml, with a minimum of 5.14 ng/ml and a maximum of 8.46 ng/ml. Group III presented the highest mean CRP levels at 7.765 ± 0.683 ng/ml, with values spanning from 6.69 to 9.30 ng/ml. The statistical analysis demonstrated a highly significant difference between the groups (p<0.001). ***(Graph No. 1)***



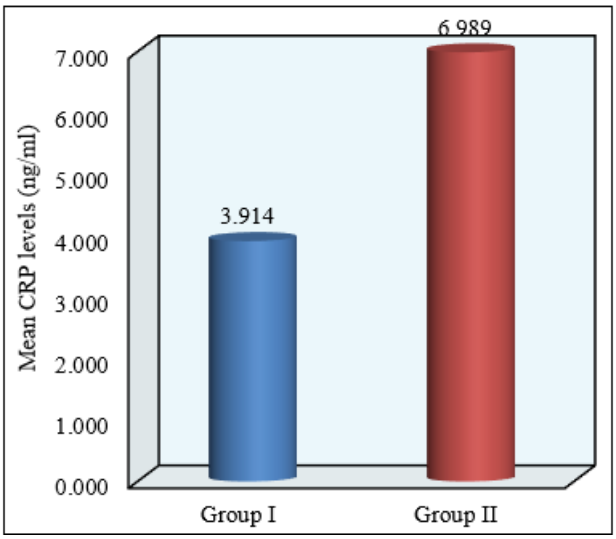
Graph 1: Mean CRP levels (ng/ml) b/w 3 groups

The multiple comparison analysis of CRP levels among the three groups revealed significant differences. Group I showed notably lower CRP concentrations when compared to both Group II and Group III. The mean difference in CRP levels between Group I and Group II was -3.075 ng/ml, with a confidence interval ranging from -3.623 to -2.527 ng/ml. Similarly, the difference between Group I and Group III was more pronounced at -3.851 ng/ml, with a confidence interval extending from -4.399 to -3.302 ng/ml. Both comparisons yielded highly significant results (p<0.001), indicating substantial differences in CRP levels (***Table 3***).

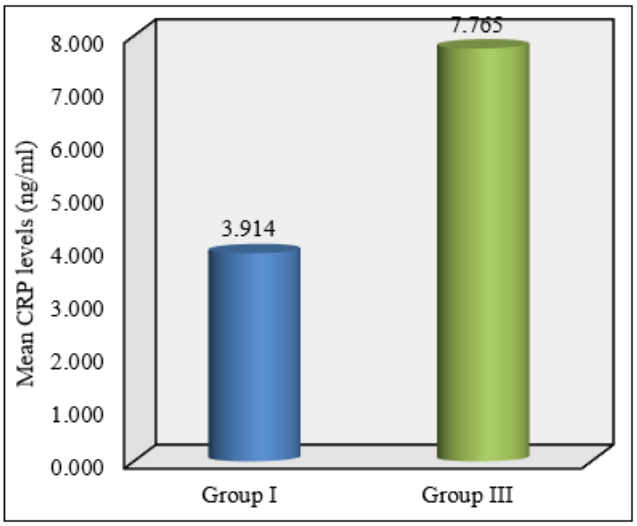
Table 3: Multiple comparison of mean diff. in CRP levels (ng/ml) b/w 3 groups using Tukey's Post hoc Test

(I) Groups	(J) Groups	Mean Diff.(I-J)	95% CI for the Diff		p-value
			Lower	Upper	
Group I	Group II	-3.075	-3.623	-2.527	<0.001*
	Group III	-3.851	-4.399	-3.302	<0.001*
Group II	Group III	-0.775	-1.324	-0.227	0.003*

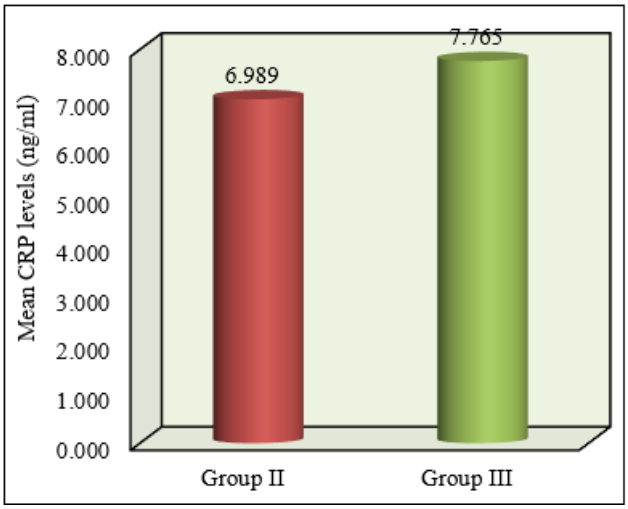
The variation between Group II and Group III was comparatively smaller, with a mean difference of -0.775 ng/ml and a confidence interval spanning from -1.324 to -0.227 ng/ml. Despite the lower magnitude, this difference remained statistically significant (p=0.003), suggesting a measurable increase in CRP levels between these groups. This infers that the mean CRP levels was significantly highest in Group III, followed by Group II and least in Group I. ***(Refer Fig no. 2 to 4)***



Graph 6: Mean CRP levels (ng/ml) b/w Group I & Group II



Graph 7: Mean CRP levels (ng/ml) b/w Group I & Group III



Graph 8: CRP levels (ng/ml) b/w Group II & Group III

The analysis of C-reactive protein (CRP) levels in serum demonstrated distinct variations across the three groups. Healthy individuals exhibited the lowest concentration of this inflammatory marker, reflecting a baseline expression associated with normal systemic conditions.

Patients with periodontitis demonstrated a notable increase in CRP levels, suggesting a heightened inflammatory state associated with periodontal disease. The elevated CRP concentrations observed in this group likely resulted from the release of pro-inflammatory cytokines, which stimulate CRP production. These findings reinforce the established link between periodontal inflammation and systemic alterations, underscoring CRP's role as a potential indicator of disease severity.

Patients with periodontitis and myocardial infarction in remission exhibited the highest CRP levels among the groups, reflecting a compounded inflammatory burden. The pronounced elevation in CRP levels within this group suggests persistent immune activation, possibly due to residual effects of myocardial infarction and ongoing periodontal disease.

These findings highlight the potential interplay between periodontal inflammation and cardiovascular health, reinforcing CRP's significance as a biomarker for both localized and systemic disease processes.

The observed trend in CRP levels across the groups emphasizes its role as a sensitive marker of inflammatory status, reflecting both periodontal and systemic disease progression.

The results suggest a clear relationship between periodontal health and systemic inflammatory response, reinforcing the necessity for interdisciplinary approaches in managing conditions that influence both oral and systemic health.

4. Figures

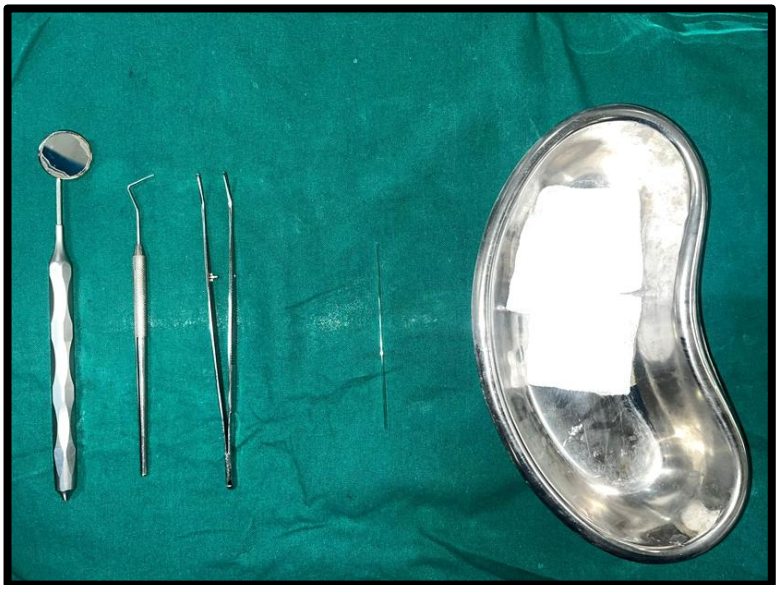


Figure 1: Armamentarium

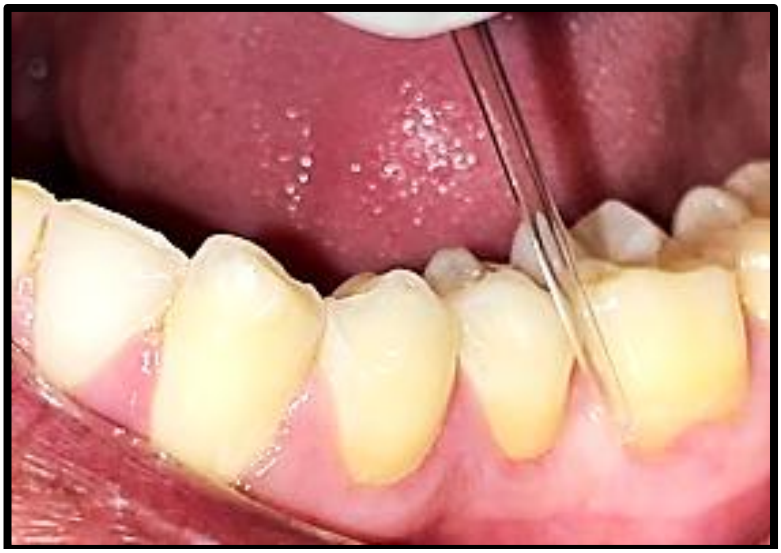


Figure 2: GCF collection method

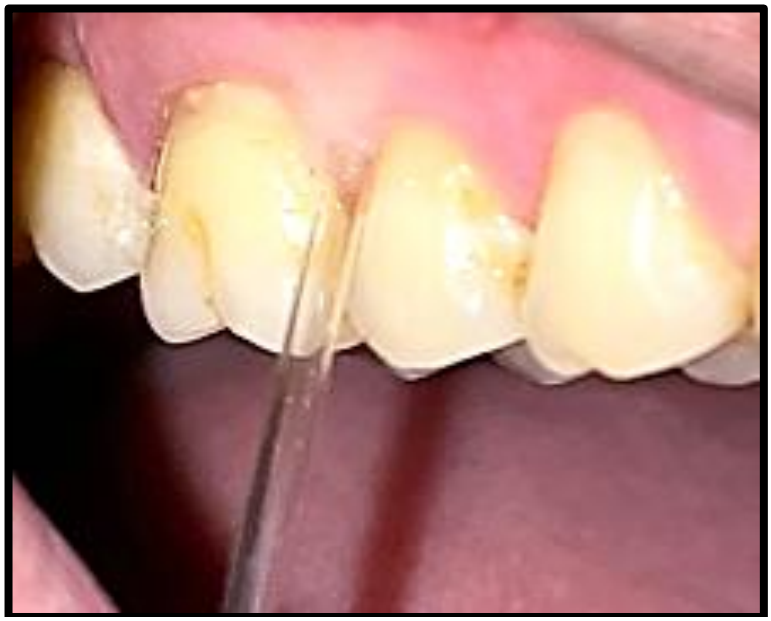


Figure 3: GCF collection method

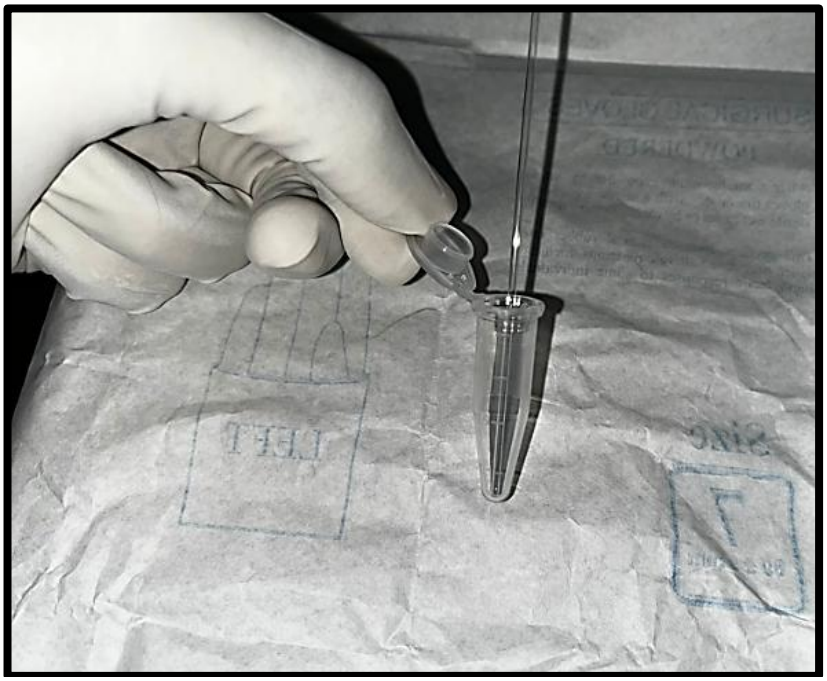


Figure 4: Transferring of GCF sample to vials

Figure 5: ELSIA kit of CRP

Figure 6: Addition of Samples

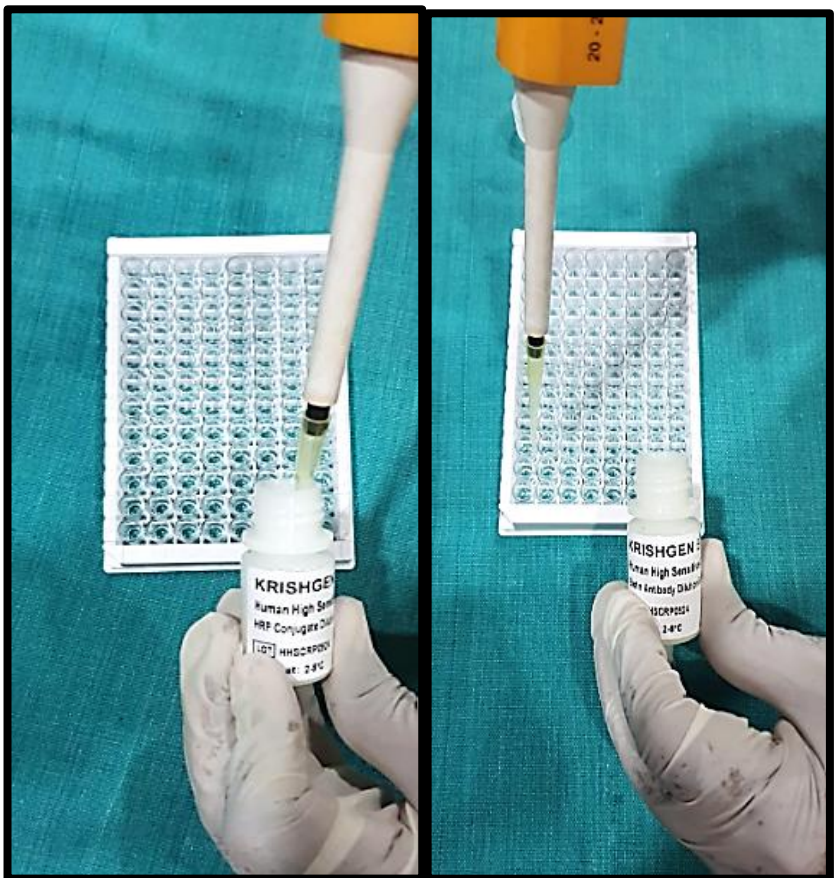


Figure 7: Addition of Streptavidin-HRP

Figure 7: Addition of TMB Substrate

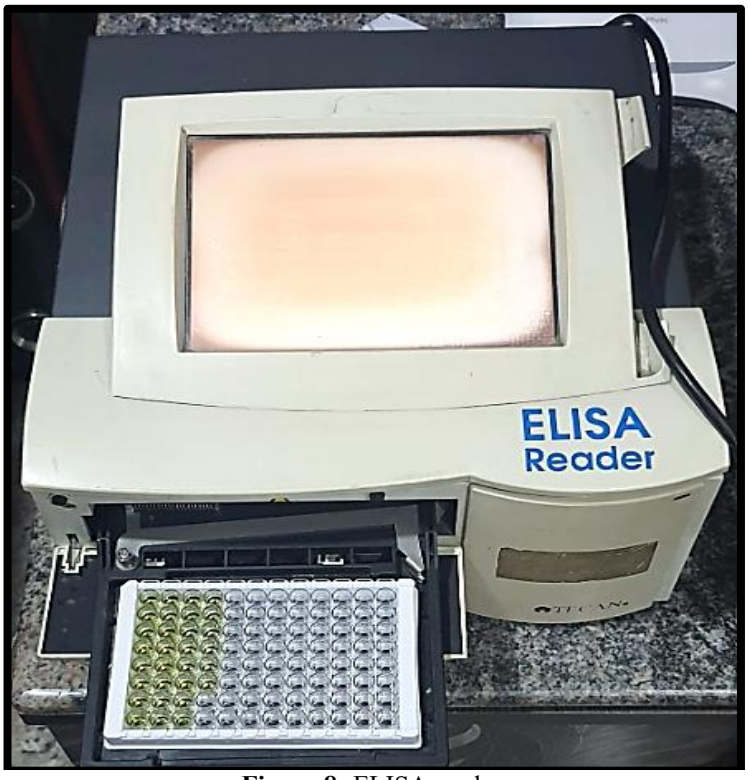


Figure 8: ELISA reader

5. Discussion

C-reactive protein (CRP) is a sensitive acute-phase protein synthesized by hepatocytes in response to inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) during systemic inflammation [19]. It plays a key role in host defense by promoting phagocytosis and activating the classical complement cascade [20]. Elevated serum CRP levels are well-established as markers for systemic inflammation and have been directly associated with an increased risk of cardiovascular events, including myocardial infarction (MI) [21, 22].

Periodontitis, a chronic multifactorial inflammatory disease caused by microbial biofilm and sustained by a dysregulated host immune response, leads to the destruction of periodontal support structures [23]. The inflammation in periodontitis extends beyond the local tissues and may

contribute to systemic inflammatory burden, as evidenced by elevated serum CRP levels in affected individuals [24]. It is postulated that inflammatory mediators from periodontal tissues may spill into systemic circulation, promoting or exacerbating systemic conditions such as atherosclerosis [25, 26].

In the present study, CRP levels were assessed in gingival crevicular fluid (GCF), which reflects local inflammatory status at the periodontal site [21,22]. The results demonstrated a statistically significant increase in CRP levels from healthy controls (Group I) to chronic periodontitis patients (Group II), with the highest levels observed in those with periodontitis and a history of MI in remission (Group III). These findings suggest that periodontal inflammation alone can elevate local CRP expression, and that the presence of a systemic inflammatory condition such as cardiovascular disease further amplifies this effect.

GCF, being a transudate of the periodontal tissues, serves as a valuable diagnostic medium for monitoring localized inflammation. Several previous studies have confirmed that CRP is detectable in GCF and is positively associated with periodontal disease severity [27,28]. Our study corroborates this evidence, showing that even in clinical remission, post-MI patients exhibit significantly elevated GCF CRP levels compared to those with periodontitis alone. This supports the hypothesis of a sustained low-grade systemic inflammatory state in such individuals, which may perpetuate or intensify local periodontal inflammation [30].

These findings align with prior research that highlighted the bidirectional relationship between periodontal disease and cardiovascular conditions. D'Aiuto et al. reported reductions in systemic CRP following periodontal therapy, suggesting a systemic impact of localized periodontal treatment [31]. Similarly, Pussinen et al. demonstrated a link between poor periodontal health and increased cardiovascular risk markers, including CRP [30].

Despite the strength of these associations, certain limitations of this study must be acknowledged [29,30]. The cross-sectional design precludes causal inference. Furthermore, serum CRP levels were not simultaneously assessed, which could have provided a more comprehensive understanding of the systemic-local inflammatory relationship [31]. Future longitudinal and interventional studies evaluating both serum and GCF biomarkers are warranted to better elucidate the mechanistic pathways connecting periodontal and cardiovascular diseases [31,32].

While these observations offer valuable insights, they also open a vista for future research. Longitudinal studies incorporating both local and systemic inflammatory markers are needed to clarify causal relationships, evaluate the impact of periodontal therapy on systemic health outcomes, and explore the potential of CRP as a predictive or therapeutic biomarker in periodontitis patients with cardiovascular comorbidities. Such investigations may ultimately contribute to more personalized and integrative models of healthcare [31,32].

6. Conclusion

Within the limitations of this study, it can be concluded that C-reactive protein (CRP) levels in gingival crevicular fluid may serve as a valuable indicator of local periodontal inflammation and may be influenced by underlying systemic conditions such as myocardial infarction [29]. The observed gradient of CRP expression across study groups underscores the importance of recognizing the periodontal-cardiovascular inflammatory link [32]. These findings emphasize the need for an integrated, multidisciplinary approach in the management of patients with coexisting periodontal and cardiovascular conditions. Further prospective studies are required to validate the prognostic value of GCF CRP and its potential role in risk assessment and therapeutic monitoring [29,30].

References

[1] Nguyen TT, Ngo LQ, Promsudthi A, Surarit R. Salivary Lipid Peroxidation in Patients with Generalized Chronic Periodontitis and Acute Coronary Syndrome. J Periodontol 2016; 87(2):134–141. doi: 10.1902/jop.2015.150353. 29.

[2] Shojaee M, Fereydooni GM, Maliji G, Bijani A, Aghajanpour MSM, Mousavi KSN. C - reactive protein levels in patients with periodontal disease and normal subjects. International journal of molecular and cellular medicine 2013; 2(3):151–155. PMID: 24551806 30.

[3] Toda M, Tsukinoki R, Morimoto K. Measurement of salivary adiponectin levels. Acta Diabetol 2007; (1):20–22. doi: 10.1007/s00592-007-0236-8.

[4] Khan SS, Ning H, Wilkins JT, Allen N, Carnethon M, Berry JD, Sweis RNN. Association of Body Mass Index with Lifetime Risk of Cardiovascular Disease and Compression of Morbidity. JAMA Cardiol 2018; 3(4):280-287. doi:10.1001/jamacardio.2018.0022

[5] El-Beshbishy HA, Maria RA, Bardi FA. Biochemical and C-reactive protein alterations in myocardial infarction periodontitis patients. Am J Med Sci 2014; 348:181–185. doi: 10.1097/MAJ.0000000000000253.

[6] Rathnayake N, Gustafsson A, Norhammar A, Kjellstrom B, Klinge B, Ryden L, Tervahartiala T.Salivary Matrix Metalloproteinase-8 and-9 and Myeloperoxidase in Relation to Coronary Heart and Periodontal Diseases: A Subgroup Report from the PAROKRANK Study (Periodontitis and Its Relation to Coronary Artery Disease). PLoS One 2015; 10: e0126370. doi: 10.1371/journal.pone.0126370.

[7] Emingil G, Atilla G, Baskesen A, Berdeli A. Gingival crevicular fluid EMAP-II, MIP-1a and MIP-1b levels of patients with periodontal disease. J Clin Periodontol 2005; 32, 880–885. doi: 10.1111/j.1600-051X.2005.00780. x.

[8] Pradeep AR, Daisy H, Hadge P. Serum levels of monocyte chemoattractant protein-1 in periodontal health and disease. Cytokine 2009; 47, 77–81. doi: 10.1016/j.cyto.2009.05.012.

[9] Afrashtehfar KI. Patient and miniscrew implant factors influence the success of orthodontic miniscrew implants. Evid Based Dent. 2016;17(4):109-110. doi: 10.1038/sj.ebd.6401202. PMID: 27980330

[10] Touyz LZG, Afrashtehfar KI. Implications of bisphosphonate calcium ion depletion interfering with desmosome epithelial seal in osseointegrated implants and pressure ulcers. Med Hypotheses. 2017;107:22-25. doi: 10.1016/j.mehy.2017.07.013. PMID: 28915955

[11] Afrashtehfar KI, Afrashtehfar CD. Lack of association between overload and peri-implant tissue loss in healthy conditions. Evid Based Dent. 2016;17(3):92-93. doi: 10.1038/sj.ebd.6401193. PMID: 27767107

[12] Touyz LZ, Afrashtehfar KI. Cryogenically salvaged teeth as a potential source for grafting dentoalveolar, periodontal or maxillofacial defects. Med Hypotheses. 2016;92:28-30. doi: 10.1016/j.mehy.2016.04.028. PMID: 27241250

[13] Afrashtehfar KI, Moshaverinia A. Five Things to Know About Regenerative Periodontal Therapies in Dental Medicine. J N J Dent Assoc. 2015;86(2):12-13. PMID: 26242103

[14] Garcia-Contreras R, Chavez-Granados PA, Jurado CA, Aranda-Herrera B, Afrashtehfar KI, Nurrohman H. Natural Bioactive Epigallocatechin-Gallate Promote Bond Strength and Differentiation of Odontoblast-like Cells. Biomimetics (Basel). 2023;8(1):75. doi: 10.3390/biomimetics8010075.PMID: 36810406

[15] Tonetti MS, D'Aiuto F, Nibali L, Donald A, Storry C, Parkar M, Suvan J. Treatment of periodontitis and endothelial function. N Engl J Med. 2007; 356(9):911–920. doi: 10.1056/NEJMoa063186

[16] Graves D. Cytokines that promote periodontal tissue destruction. J. Periodontol. 2008;79(8): 1585–1591. doi: 10.1902/jop.2008.080183

[17] Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: framework and proposal of a new classification and case definition. Journal of Periodontology 2018; 89 (Suppl 1): S159–S172, 2018. doi: 10.1002/JPER.18-0006.

[18] Schenkein HA, Loos BG. Inflammatory mechanisms linking periodontal diseases to cardiovascular diseases. J Clin Periodontol. 2013; 14(0 14): S51-69. doi: 10.1111/jcpe.12060.

[19] Andriankaja OM, Genco RJ, Dorn J, Dmochowski J, Hovey K, Falkner KL, Scannapieco F. The use of different measurements and definitions of periodontal disease in the study of the association between periodontal disease and risk of myocardial infarction. J Periodontol 2006; 77(6):1067-1073. doi: 10.1902/jop.2006.050276.

[20] Bhavsar I, Miller C, Al-Sabbagh M. Macrophage Inflammatory Protein-1 Alpha (MIP-1 alpha)/CCL3: As a biomarker. 2015; 223-249 Doi:10.1007/978-94-007-7696-8_27

[21] Obata Y, Kita S, Koyama Y, Fukuda S, Takeda H, Takahashi M, Fujishima Y. Adiponectin/T-cadherin system enhances exosome biogenesis and decreases cellular ceramides by exosomal release, JCI Insight 2018; 19;3(8): e99680. doi: 10.1172/jci.insight.99680.

[22] Denzel MS, Scimia MC, Zumstein PM, Walsh K, Ruiz-Lozano P, Ranscht B. Tcadherin is critical for adiponectin-mediated cardioprotection in mice. J. Clin. Investig. 2010; 120(12):4342-52. doi: 10.1172/JCI43464.

[23] Joshi, C., Bapat, R., Anderson, W., Dawson, D., Hijazi, K. and Cherukara, G., 2021. Detection of periodontal microorganisms in coronary atheromatous plaque specimens of myocardial infarction patients: A systematic review and meta-analysis. *Trends in cardiovascular medicine*, *31*(1), pp.69-82.

[24] Caton, J.G., Armitage, G., Berglundh, T., Chapple, I.L., Jepsen, S., Kornman, K.S., Mealey, B.L., Papapanou, P.N., Sanz, M. and Tonetti, M.S., 2018. A new classification scheme for periodontal and peri-implant diseases and conditions–Introduction and key changes from the 1999 classification. *Journal of periodontology*, *89*, pp.S1-S8.

[25] Tonetti MS, Van Dyke TE. Working Group 1 of the Joint EFP/AAP Workshop. Periodontitis and atherosclerotic cardiovascular disease: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. J Periodontol 2013; 84(4 Suppl): S24-S29. doi: 10.1902/jop.2013.1340019.

[26] Syndergaard B, Al-Sabbagh M, Kryscio RJ, Xi J, Ding X, Ebersole JL, Miller CS. Salivary biomarkers associated with gingivitis and response to therapy. J Periodontol 2014; 85(8): e295–e303. doi: 10.1902/jop.2014.130696.

[27] Miller CS, Foley JD, Bailey AL, Campell CL, Humphries RL, Christodoulides N, Floriano PN. Current developments in salivary diagnostics. Biomark Med. 2010; 4(1):171–189. doi: 10.2217/bmm.09.68

[28] Zhang Y, Kang N, Xue F, Qiao J, Duan J, Chen F, Cai Y. Evaluation of salivary biomarkers for the diagnosis of periodontitis. BMC Oral Health. 2021; 21(1):266. doi: 10.1186/s12903-021-01600-5.

[29] Gupta M, Chaturvedi R, Jain A. Role of cardiovascular disease markers in periodontal infection: understanding the risk. Indian J Dent Res 2015; 26(3):231-236. doi: 10.4103/0970-9290.162873.

[30] Jotwani R, Eswaran SV, Moonga S, Cutler CW. MMP-9/TIMP-1 imbalance induced in human dendritic cells by Porphyromonas gingivalis. FEMS Immunol Med Microbiol 2010; 58(3):314–321. doi: 10.1111/j.1574-695X.2009.00637. x.

[31] Henson BS, Wong DT. Collection, storage, and processing of saliva samples for downstream molecular applications. Methods Mol Biol. 2010; 666:21–30. doi: 10.1007/978-1-60761-820-1_2. Kindstedt, E., Johansson, L., Palmqvist, P., Koskinen Holm, C., Kokkonen, H., Johansson, I., Rantapää Dahlqvist, S. and Lundberg, P., 2018. Association between marginal jawbone loss and onset of rheumatoid arthritis and relationship to plasma levels of RANKL. *Arthritis & Rheumatology*, *70*(4), pp.508-515.

[32] Lee, E., Ho, Y.P., Ho, K.Y., Tsai, C.C. and Yang, Y.H., 2003. Potential role of vascular endothelial growth factor, interleukin-8 and monocyte chemoattractant protein-1 in periodontal diseases. *The Kaohsiung journal of medical sciences*, *19*(8), pp.406-414.