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Serum C-Reactive Protein as a Biomarker of Periodontal Disease

A Comparative Study of ELISA and Latex Agglutination

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Abstract

This study aimed to compare two laboratory methods, enzyle-linked immunosorbent assay (ELISA) and the latex agglutination test, for the detection of serum C-reactive protein in individuals with different periodontal health statuses. Inn addition, the study evaluated the association between CRP levels, periodontal disease severity, age, and gender. A case-control study including 90 participants (30 healthy, 30 with gingivitis, and 30 with periodontitis was performed. Clinical periodontal examinations were done, and serum samples were collected for CRP analysis by latex agglutionation rtest and ELISA. Chi-square, one-way ANOVA, and Tukey post hoc analyses were performed to compare CRP levels among the study groups and to determine correlations between CRP levels, disease severity, age, and gender. Serum CRP levels increased significantly with periodontal disease severity ($P < 0.001$). ELISA detected higher CRP concentrations from healthy controls than from individuals with periodontitis, while the latex agglutination test showed high specificity (96.2%) but low sensitivity (27%) for CRP detection. The elevation was associated with increasing disease severity, indicating its potential use as a biomarker of periodontal inflammation. Age showed a significant association with disease severity, whereas geneder was not significantly associated. Serum CRP levels increased significantly with periodontal disease severity. Although the latex agglutination test demonstated high specificity abd ay serve as a rapid screenkng method, ELISA provided superior sensitivity and quantitative accuracy, making it the preferred method for clinical and research applications.

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Introduction

Periodontal disease is a multifactorial and chronic inflammatory condition, which is caused due to an imbalance within the dental plaque and involves progressive destruction of the supporting structures of teeth, such as the gingiva, periodontal ligament, cementum, and alveolar bone. Periodontal disease is caused by the interaction of bacteria and the host immune response, causing destruction of connective tissue and lead eventually to alveolar bone resorption [1,2]. Beyond its local effects, periodontal disease has been increasingly recognized as a potential contributor to systemic inflammation. During periodontal infection, bacterial products and inflammatory mediators may enter the systemic circulation, stimulate the hepatic production of acute-phase proteins and amplify the systemic inflammatory response. Consequently, periodontitis has been associated with elevated circulating levels of inflammatory biomarkers that have been implicated in several chronic systemic conditions, including diabetes mellitus, rheumatoid arthritis and cardiovascular disease [2,3].

Among these biomarkers, one of the acute-phase proteins that has been studied the most is C-reactive protein (CRP). Hepatocytes are the main producers of CRP in response to pro-inflammatory cytokines, especially interleukin-6 (IL-6). CRP is crucial for innate immunity because it activates complement, opsonizes microbes, and controls inflammatory processes. Serum CRP concentrations increase rapidly during acute and chronic inflammatory conditions and decrease following the resolution of inflammation, making CRP a reliable indicator of systemic inflammatory activity [2,4,5].

Moreover, greater CRP levels are associated with increased probing pocket depth, clinical attachment loss, and bleeding on probing, hence confirming its potential as a biomarker for periodontal disease activity and progression. Several clinical studies have shown significantly higher serum CRP concentrations in patients with periodontitis compared with periodontally healthy individuals, indicating that CRP may reflect both periodontal inflammatory burden and disease severity [6,7].

Accurate measurement of CRP is therefore important for both clinical practice and research. Several laboratory techniques are available for CRP determination, including immunoturbidimetric assays, latex agglutination tests, and enzyme-linked immunosorbent assay (ELISA). ELISA is well recognized as a highly sensitive and quantitative immunoassay with exceptional analytical precision that can identify low amounts of CRP. In contrast, the latex agglutination test is a rapid, simple, and cost-effective qualitative or semi-quantitative method that can be performed with minimal laboratory infrastructure, making it particularly suitable for routine clinical screening and resource-limited settings [8,9].

Although numerous investigations have evaluated CRP as a biomarker of periodontal inflammation, relatively few studies have directly compared the clinical applicability of ELISA and latex agglutination for CRP assessment across different periodontal conditions, particularly in Middle Eastern populations. In addition, limited evidence is available regarding the relationship between CRP concentrations, periodontal disease severity, and demographic variables such as age and gender within the same study population. Therefore, the present case-control study aimed to compare serum CRP levels measured by ELISA and the latex agglutination test among periodontally healthy individuals and patients with gingivitis or periodontitis. The study also examined the association between serum CRP concentrations, periodontal disease severity, age, and gender to evaluate the potential clinical value of CRP as a biomarker of periodontal inflammation.

Material and Methods

A case-control study was conducted to investigate the association between serum CRP levels and periodontal disease among adults aged 20–60 years. A total of 90 participants were recruited from different private dental clinics. According to their periodontal condition, participants were allocated into three equal groups (n = 30 per group): a healthy control group, a gingivitis group, and a periodontitis group. The diagnosis and classification of periodontal conditions were established according to the 2018 Classification of Periodontal and Peri-Implant Diseases and Conditions. Clinical periodontal assessment included the Plaque Index, Bleeding Index, Probing Pocket Depth, and Clinical Attachment Loss. Healthy subjects with normal periodontium were included in the control group, while subjects with either gingivitis or periodontitis were grouped based on their clinical conditions. Criteria for inclusion in the study were patients who were systemically healthy individuals without any prior exposure to antibiotics in the previous 6 months, any prior treatment in their periodontium within the previous months, the presence of any mucosal lesions, any intake of drugs affecting periodontal tissues, and no prior smoking habit. This was done to reduce the impact of any confounders that may affect the serum level of CRP independent of periodontitis. Post hoc power analysis was performed using G*Power 3.1.9.7. Based on a one-way ANOVA with three groups, a total sample size of 90 participants, an alpha level of 0.05, and the observed effect size (Cohen's f = 1.10), the achieved statistical power (1 − β) was >0.999, indicating that the study was adequately powered to detect differences in serum CRP levels among the study groups.

Two immunological procedures were used to assess serum C-reactive protein levels: the latex slide agglutination test and ELISA. Initially, all serum specimens were evaluated using the latex slide agglutination technique, a quick qualitative immunoassay that relies on the visual agglutination of latex particles coated with anti-human CRP antibodies. The formation of visible agglutination indicated the presence of CRP at or above the assay's detection threshold, whereas the absence of agglutination was interpreted as a negative result (Figure 1).

The levels of CRP concentration in the serum were then evaluated for each sample using a commercial ELISA test kit in compliance with the manufacturer's instructions. ELISA is a very sensitive immunoassay that measures CRP quantitatively using an antigen-antibody reaction. Enzymatic colorimetric detection is also used. The optical density was measured using a microplate reader, and the CRP concentrations were determined using a calibration curve based on CRP standards. The use of a latex agglutination test followed by ELISA made it possible to compare a rapid qualitative technique with a quantitative laboratory procedure for determining CRP levels [9].

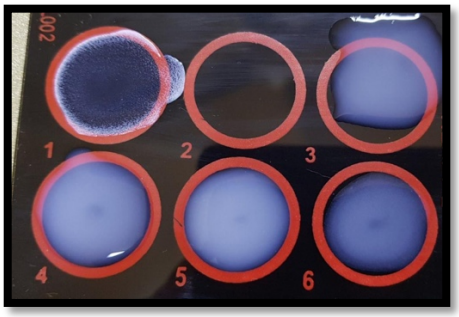

Figure 1. Latex agglutination slide "1- positive agglutination; 3-4-5-6- negative agglutination."

Statistical analyses were carried out using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). Categorical data were reported as frequencies and percentages, whereas continuous variables were presented as mean ± SD. Differences in mean CRP levels and age across study groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test for pairwise comparison. The Pearson chi-square test was used to assess the associations between categorical variables. Statistical significance was defined as a P-value of <0.05.

Results

Ninety participants were participated in the study, evenly divided into three groups (n = 30 each): healthy controls, gingivitis patients, and periodontitis patients. The demographic features of the participants are illustrated in (Figure 2). Gender distribution was comparable among the groups, Pearson's Chi-square analysis revealed no significant association between gender and gingival health status ($\chi^2 = 4.286$, df = 2, P = 0.117). Therefore, the distribution of periodontal conditions was independent of gender within the study population. The mean age increased progressively across the study groups, with the highest mean age observed in the periodontitis group (Figure 3).

The prevalence of positive CRP differed significantly among the study groups, as shown in (Table 1). The periodontitis group showed the highest frequency of CRP positivity (21/30, 70.0%), followed by the Gingivitis group (5/30, 16.7%) and the Control group (2/30, 6.7%). A chi-square analysis demonstrated a highly significant association between study group and CRP status ($\chi^2 = 31.36$, df = 2, $P < 0.001$). Pairwise comparisons showed that the Periodontitis group had significantly higher CRP positivity than both the Gingivitis and Control groups ($P < 0.001$ for both comparisons), whereas the difference between the Gingivitis and Control groups was not statistically significant ($P > 0.05$). Table-2 and Figure-4 illustrate this.

Serum concentrations of C-reactive protein CRP measured using ELISA increased gradually

among the study groups. The mean CRP concentration was significantly lower among healthy controls (0.98 ± 0.32 mg/L), higher among patients with gingivitis (1.40 ± 0.66 mg/L), and highest among periodontitis patients (2.95 ± 1.14 mg/L) (Table 3). Significant variations in the mean blood CRP concentrations among all research groups were shown by the post hoc Tukey's multiple comparison test (Table-4). In comparison to the gingivitis and periodontitis groups, the healthy group had significantly lower CRP levels (mean difference = 0.42 mg/L, P = 0.021; and 1.97 mg/L, P < 0.001, respectively). Additionally, compared to the gingivitis group, the periodontitis group had significantly higher CRP levels (mean difference = 1.55 mg/L, P < 0.001).

Table 6 shows the diagnostic accuracy of the latex agglutination test for serum CRP when compared with ELISA. The sensitivity of the latex agglutination assay was 27.0%. This shows that about a quarter of study participants with raised CRP detected by ELISA also had a positive latex result. Specificity of the latex assay was 96.2%.

The positive predictive value (PPV) of the latex assay was 83.3%. This means that most study participants with a positive latex result were correctly classified and had raised CRP concentrations by ELISA standards. On the other hand, the negative predictive value was 65.4%. This means that about a third of participants with negative latex test results were false negatives by ELISA standards. Overall, the latex agglutination correctly classified about two-thirds of all participants (accuracy = 67.8%). The Cohen's kappa coefficient (κ = 0.259) showed fair agreement between the latex agglutination method and ELISA.

Discussion

This research has assessed the usefulness of CRP as a marker for the diagnosis of periodontal disease using two different methods of measurement: ELISA and latex agglutination. The results indicated that the positive rate of CRP was significantly higher in periodontitis than in gingivitis and in healthy individuals. This result has supported the hypothesis that the level of systemic inflammatory markers correlates with the intensity of inflammation of periodontal tissue. This finding confirms that periodontitis should be regarded not only as an infection but also as a systemic inflammatory disease.

The current investigation showed that as periodontal disease severity increased, CRP positivity as measured by the latex agglutination test gradually increased. Positive latex responses were seen in only 6.7% of healthy individuals, but they increased to 16.7% in gingivitis patients and 70.0% in periodontitis patients. This pattern indicates that when periodontal disease worsens, systemic inflammatory activity increases, raising circulating CRP levels that the latex agglutination test may detect.

Likewise, the quantitative analysis using ELISA also showed a gradual increase in CRP levels in the sera of the groups being studied. The healthy group had the least mean CRP level at 0.98 ± 0.32 mg/L; next is the gingivitis group with a CRP level of 1.40 ± 0.66 mg/L, and the greatest level of CRP was found in the group of patients suffering from periodontitis at 2.95 ± 1.14 mg/L. The mean level of CRP in the periodontitis group was around three times greater than in the healthy control subjects and more than twice that of the gingivitis group.

A very significant difference was observed when all three groups were compared together (P < 0.001). the parallel increase in latex positivity and ELISA measured CRP concentrations suggests that both methods reflect similar trends in periodontal disease severity; however, only fair agreement was observed between the two assays (Cohen's κ = 0.259).

There was a statistically significant difference (P < 0.001) among the three groups considered in the overall comparison. The correlation between increasing positive results of the latex test and CRP concentration determined by ELISA demonstrates a high degree of concordance between qualitative and quantitative tests. These results are in accordance with data from the literature that showed the clinical significance of CRP in periodontal disease [6], which noted a statistically significant relationship between the CRP level in the serum and the presence of periodontitis and proved that increased CRP means an active inflammatory process in periodontal tissues. In the same way, Sawhney et al. (10), who studied the salivary CRP, found statistically significant CRP levels in the saliva of patients with periodontal disease compared with healthy subjects. CRP can bind phosphoethanolamine and phosphocholine from degraded bacterial and host cell membranes, chromatin, small nuclear ribonucleoproteins, laminin, and fibronectin in the presence of calcium [11]. When CRP binds with the mentioned ligands, it activates the complement cascade. CRP receptors are located on the surface of macrophages, monocytes, and neutrophils; hence, bound CRP targets the bacteria and damaged host cells for phagocytosis and helps in amplification of subsequent inflammatory responses. That is why the level of CRP is increased in periodontitis compared with gingivitis and healthy people.

Nevertheless, in previous studies [12,13], the association between periodontitis and CRP levels has not always been shown. For example, Noack et al. showed that the level of CRP in people suffering from periodontitis is higher than the same parameter in healthy subjects, but the strength of the correlation was rather weak, and considerable overlap was found. The authors have claimed that many factors affect CRP levels in blood circulation, and thus it cannot be claimed that its increased levels were solely the result of periodontitis development. In another study carried out by Slade et al., it was found that periodontitis has an impact on systemic inflammation but that the correlation between periodontitis and CRP levels becomes considerably lower once adjusted for smoking status, obesity, and diabetes [13].

The variations in findings from these studies compared to this one could be attributed to some methodological issues. First, the study had clearly defined healthy individuals, those with gingivitis, and periodontitis patients who did not suffer from any other systemic inflammatory conditions, which might increase the concentration of CRP in the blood. Also, the measurement of CRP level was done using ELISA, which is a very sensitive quantitative method and thus can detect even slight changes in the protein level in the bloodstream.

Regarding gender, there was no statistically significant relationship between the condition of periodontal health and gender (P = 0.117). It means that the results of the laboratory test do not depend on the gender of the patient. Our results correlate with the systematic review where it is stated that gender issues are usually associated with behaviors like smoking and personal oral hygiene. Likewise, in another study that examined 55 patients (27 men and 28 women), it was revealed that salivary CRP significantly increases in the case of chronic and aggressive periodontitis regardless of the gender of the patient [14,15].

A statistically significant correlation was found between advancing age and disease severity (P = 0.009). The higher mean age in the periodontitis group (45.4 years) compared to the control group (28.67 years) aligns with biological principles in Newman et al. [16]. This suggests that aging is associated with physiological changes in the periodontium that may increase susceptibility to inflammatory destruction. This association may reflect cumulative exposure to periodontal risk factors over time, age-related alterations in immune function, and diminished tissue repair capacity, all of which contribute to disease progression. By incorporating this perspective, our study supports that the elevated levels of CRP in the older periodontal inflammation group are not coincidental but rather reflect age-related changes in the inflammatory response.

The diagnostic performance comparison analysis demonstrated that the latex agglutination test exhibited high specificity (96.2%) but low sensitivity (27.0%) for detecting elevated serum CRP when compared with ELISA. These findings indicate that a positive Latex result is highly reliable for confirming elevated CRP, whereas a negative result cannot reliably exclude increased CRP concentrations. The high positive predictive value (83.3%) further supports the usefulness of latex agglutination as a rapid and inexpensive screening method. However, the moderate negative predictive value (65.4%), overall accuracy (67.8%), and fair agreement with ELISA (Cohen's κ = 0.259) suggest that latex agglutination should not replace quantitative ELISA, particularly when accurate assessment of low-grade systemic inflammation is required.

The relatively poor sensitivity observed in the current study is consistent with the analytical characteristics of latex agglutination assays, which generally have higher detection limits than ELISA. Consequently, individuals with mild elevations in CRP, such as patients with gingivitis or early periodontal disease, may yield negative latex results despite having detectable increases in CRP by ELISA. Our results are consistent with other reports that emphasized that highly sensitive immunoassays like ELISA detect small increases of CRP that are usually missed by classical agglutination methods [17]. Other study also found that high-sensitivity quantitative assays enable better detection of low-grade systemic inflammation and are therefore the preferred assays for clinical and research applications in chronic inflammatory diseases [17,18]. In contrast, the good specificity observed in the present study supports previous reports that the latex agglutination test is still useful as a rapid screening assay, as the positive results are highly reliable. Shivananda et al. reported that latex agglutination had higher specificity in the detection of CRP but lower sensitivity than quantitative immunoassays and suggested that it should be used as an initial screening test but not as a final quantitative method [19]. Conversely, some investigators found better correlation between the latex agglutination and automated immunoturbidimetric CRP assays, particularly with markedly elevated CRP concentrations. Burtis and Bruns note that qualitative Latex assays work well in patients with moderate or severe inflammatory responses but tend to lose their reliability at lower CRP concentrations [20], which may justify the limited sensitivity found in the present study.

The study's findings demonstrate that latex agglutination is highly specific qualitative assay for detecting elevated serum CRP; however, its limited sensitivity restricts its ability to identify mild elevations in CRP. however, ELISA is the preferred reference method because of its significantly higher analytical sensitivity and capacity to measure low CRP concentrations accurately.

Conclusion

The results of this study confirm the validity of serum CRP as a biomarker for periodontal inflammation and the gradual rise in CRP levels from individuals in good health to those with gingivitis and periodontitis. Although the latex agglutination test and the ELISA could both indicate the severity of the condition, the ELISA's analytical sensitivity and quantitative accuracy were superior. The latex agglutination test is more helpful as a quick screening test than as a conclusive diagnostic test due to its poor sensitivity, while having a very high specificity and a high positive predictive value. The results emphasize the clinical importance of measuring serum CRP in periodontal disease and suggest ELISA as the optimum laboratory technique for precise systemic inflammatory assessment.

Study limitation

There are some limitations of the study. The data cannot be generalized to a larger population due to the small sample size and case-control nature of this study. Additionally, participants were from a small geographic area and only serum CRP was studied as an inflammatory biomarker. Thus, the confirmation of these results is recommended in larger multi-geographical prospective studies to megastructures further inflammatory biomarkers.

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Conflict of Interest

The authors declare no conflict of interest.

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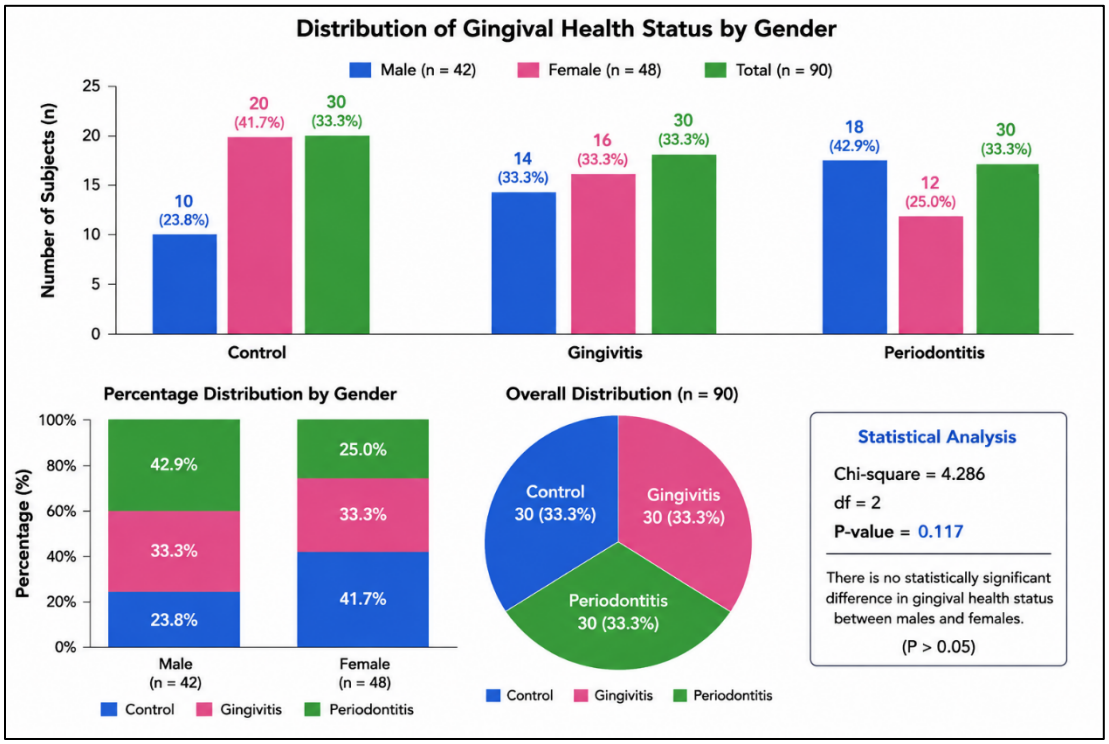

Figure 2. Distribution of gingival health status.

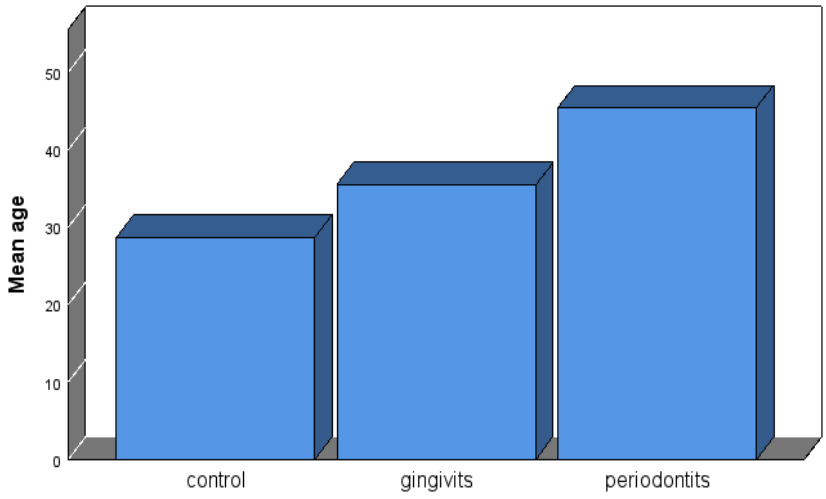

Figure 3. Mean age of study group.

Table 1. Distribution of CRP status among the study groups by Latex agglutination test.

Variable	Periodontitis (n=30)	Gingivitis (n=30)	Control (n=30)	χ^2	*P* value
CRP Positive, n (%)	21 (70)	5 (16.7)	2 (6.7)	31.36	<0.001
CRP Negative, n (%)	9 (30)	25 (83.3)	28 (93.3)		

Table 2. Comparison of CRP positivity among study group using latex agglutination test.

Comparison	CRP Positive (%)	Statistical significance
Periodontitis vs Gingivitis	70 vs 16.7	P < 0.001
Periodontitis vs Control	70 vs 6.7	P < 0.001
Gingivitis vs Control	16.7 vs 6.7	P > 0.05

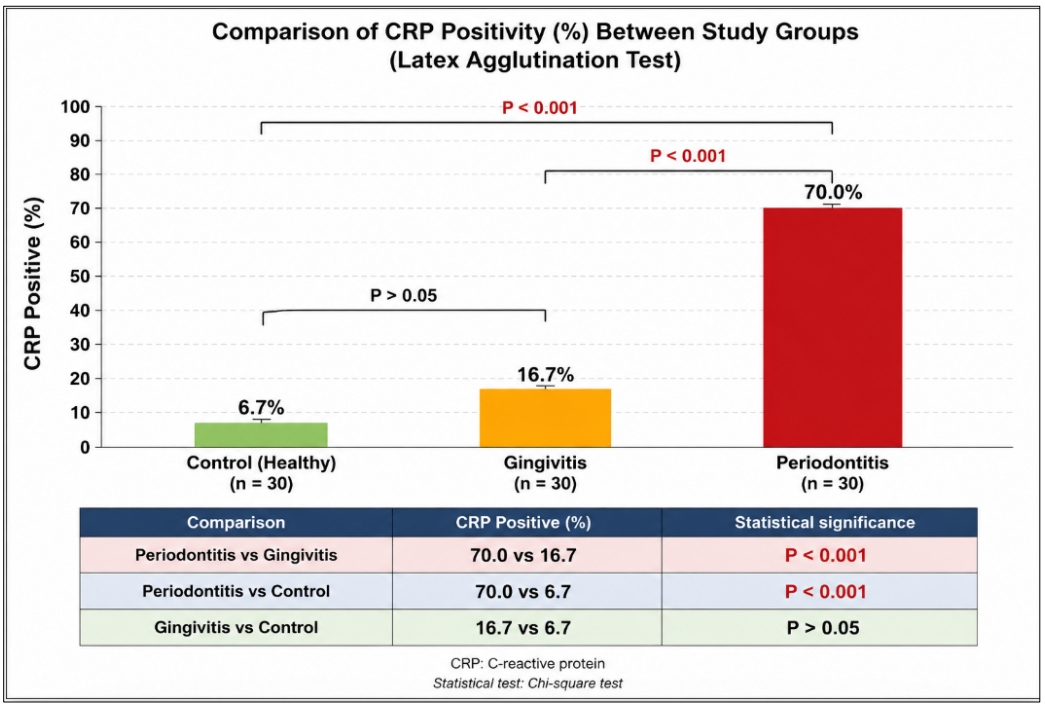

Figure 4. Comparison of CRP positivity between group.

Table 3. Serum CRP levels measured by ELISA among the study groups.

Study Group	n	Mean ± SD (mg/L)
Healthy	30	0.98 ± 0.32
Gingivitis	30	1.4 ± 0.66
Periodontitis	30	2.95 ± 1.14

Table 4. Comparison of serum CRP levels measured by ELISA among the study groups.

Comparison	Mean Difference (mg/L)	P-value
Healthy vs Gingivitis	0.42	0.021
Healthy vs Periodontitis	1.97	<0.001
Gingivitis vs Periodontitis	1.55	<0.001

Table 5. Serum CRP detection by latex agglutination and ELISA across the study groups.

Study Group	Latex Positive n (%)	Latex Negative n (%)	ELISA CRP (Mean ± SD, mg/L)	P-value*
Healthy (n = 30)	2 (6.7)	28 (93.3)	0.98 ± 0.32	<0.001
Gingivitis (n = 30)	5 (16.7)	25 (83.3)	1.4 ± 0.66	
Periodontitis (n = 30)	21 (70)	9 (30)	2.95 ± 1.14	

Latex agglutination results were compared using Pearson's Chi-square test.
ELISA CRP concentrations were compared using one-way ANOVA followed by Tukey's post hoc test.

Table 6. Diagnostic performance of the latex agglutination test using ELISA as the reference standard.

Diagnostic Parameter	Value
Sensitivity	27%
Specificity	96.2%
Positive Predictive Value (PPV)	83.3%
Negative Predictive Value (NPV)	65.4%
Accuracy	67.8%
Cohen's κ	0.259 (Fair agreement)