Clinico-Biochemical Evaluation of CRP and Complete Blood Count in Chronic Periodontitis

Kamalkishor Mankar1 and Pranjali Bawankar2*

1Reader, Department of Periodontics and Implantology, VSPM Dental College and Research Centre, Nagpur, India
2Senior Lecturer, Department of Periodontics and Implantology, VSPM Dental College and Research Centre, Nagpur, India

*Corresponding Author: Pranjali Bawankar, Senior Lecturer, Department of Periodontics, VSPM Dental College and Research Centre, Nagpur, India.

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Abstract

Introduction: A strong association of chronic periodontal disease and cardiovascular disorders has been established. The interlink being chronic inflammation. Serum levels of CRP have been shown to be a strong predictor of the risk of cardiovascular events. The relation of anemia and chronic inflammatory diseases has been reported. In light of the above-mentioned facts the present study was formulated to evaluate serum levels of CRP, Hb%, WBC count and RBC count in patients with chronic periodontitis.

Objective: To compare and correlate the serum levels of C-reactive protein and WBC, RBC, Hb% in patients with chronic periodontitis and healthy controls.

Material and Methods: A total of 120 patients equally divided into four groups depending upon the severity of chronic periodontitis (CP) were recruited for the study. The serum levels of C-reactive protein were assessed using Enzyme Linked Immunosorbant Assay. The complete blood count including WBC, RBC, Hb% was obtained using CBC cell counter.

Results: The serum CRP and WBC levels were found to be elevated in severe CP patients as compared to moderate and mild CP patients. The RBC count and Hb% was found to be lowered in severe CP as compared to other groups.

Conclusion: Within the limitations of the study it can be conclude that the serum CRP levels increases with the severity of periodontal disease The WBC count also was found to be increased in severe chronic periodontitis cases thereby suggesting active inflammation. Whereas the RBC count and Hb count significantly decreases with the severity of periodontitis thereby increasing risk of anemia.

Keywords: Chronic Periodontitis; Cardiovascular Diseases; Anemia; Inflammation; Periodontal Disease

Introduction

Periodontal diseases are infections characterized by inflammation and destruction of the supporting tissues of the affected teeth [1]. Gram-negative anaerobes are present in large numbers in subgingival dental plaque in periodontal pockets. Endotoxin, derived from Gram-negative microorganisms, induces high levels of acute-phase proteins after its interaction with receptors expressed on the surface of neutrophils and monocytes, which are present in large numbers in periodontal inflammation [2]. Inflamed and ulcerated subgingival pocket epithelium forms an easy port of entry for dental plaque bacteria. For more than a century it has been postulated that infectious agents are responsible for atherosclerotic diseases [3]. The association between atherosclerosis and infections seems to be a rational one, as the process of development of atherosclerosis involves a chronic low grade inflammation [4]. Moreover, systemic markers of inflamm-
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mation (that is C-reactive protein, leukocyte count, fibrinogen, cell-adhesion molecules and proinflammatory cytokines) are predictors of present and future cardiovascular events and disease [5].

Recent research has identified prominent role of inflammation in atherosclerosis and its complications [6]. Infectious agents have emerged as potential risk factors for coronary heart disease (CHD), with the possibility that persistence of infection is related to the degree of inflammation and the severity of atherosclerosis [7]. Recent epidemiological studies have shown that levels of acute-phase proteins, including C-reactive protein (CRP), are increased in otherwise healthy adults with poor periodontal status [8] Increased serum levels of CRP have been shown to be a strong predictor of the risk of cardiovascular events [9].

The White blood cell (WBC) count which rises during infections and inflammatory illness, predicts coronary heart disease (CHD) morbidity and mortality in large scale community studies [10,11] and among patient at risk for CHD, independent of traditional cardiovascular risk factors [12].

IL-6 stimulates release of neutrophil leukocytes from bone marrow into circulation, as a part of acute and chronic inflammatory reactions [13]. Increased total leukocytes count may promote atherosclerosis, thrombosis, and ischemia through several potential mechanisms, including microvascular occlusion by activated neutrophils and monocytes under ischemic conditions [14]. Meta-analysis have shown that leukocytes count is a significant predictor of CHD risk, independent of classic coronary risk factors [15]. It has been investigated in several studies whether numbers of leukocytes are elevated in periodontitis compared to healthy subjects without periodontal disease. A study by Hutter, et al. [16] showed that periodontitis patients have a lower hematocrit value. In the subsequent analysis it was seen that the lower hematocrit in periodontitis patients was explained by lower numbers of erythrocytes in their blood. Consequently, a lower level of hemoglobin (Hb) also was observed in periodontitis.

It has been established in the past decade that acute phase proteins not only appear in acute and severe disease processes, but also in longstanding, chronic conditions like chronic periodontitis. Slightly elevated levels of CRP were determined to have a predictive value for the occurrence of a cardiovascular event [17]. Since, periodontitis is a chronic inflammatory and infectious disease, CRP levels in patients with periodontitis might have some predictive value for the occurrence of cardiovascular diseases. There is an increasing number of reports which have studied CRP in relation to periodontitis. Epidemiological studies from Buffalo reported elevated CRP in blood plasma among subjects with severe periodontal attachment loss in comparison to individuals with minimal or no attachment loss [18,19]. In light of above mentioned aspects of relationship between periodontal disease and changes in serum levels of CRP, Hb% WBC count (TLC and DLC) and RBC count and paucity of sufficient literature in this regard, a study was intended to be carried out to estimate the serum levels of CRP, Hb% WBC count, and RBC count in patients with chronic periodontitis.

Materials and Methods

A total of 120 patients were selected for the study from out patient department of Periodontics, of our institute. Thirty patients each for mild, moderate, and severe chronic generalized periodontitis based on Criteria given by AAP International Workshop for Classification of Periodontal Diseases, 1999 served as test groups. Thirty subjects with healthy periodontium formed the control group with no evidence of any periodontal pathology clinically and any history of systemic disease.

Inclusion criteria

1. Patients diagnosed with chronic periodontitis and categorized as mild moderate and severe depending upon the probing pocket depth, clinical attachment level.
2. Systemically healthy patients
3. Patients who did not undergo any periodontal therapy in last 2 years.

Exclusion criteria

1. History of systemic disease (Diabetes, Cardiovascular diseases).
2. Subjects who had taken any medication (steroids, antibiotics, anti-allergic drugs) for at least 6 months prior to the sampling procedure.
3. Subjects who had undergone oral prophylaxis or extraction in the last six months.
4. Individuals with smoking habit.

**Group-A [chronic mild periodontitis]**: Consisted of 30 subjects with 1 to 2 mm of clinical attachment loss and bleeding on probing.

**Group-B [chronic moderate periodontitis]**: Consisted of 30 subjects with 3 to 4 mm of clinical attachment loss and bleeding on probing.

**Group-C [chronic severe periodontitis]**: Consisted of 30 subjects with ≥ 5 mm of clinical attachment loss and bleeding on probing.

Under aseptic conditions 2 ml of blood was collected from the antecubital fossa by venepuncture with 24-gauge needle using 2 ml sterile disposable syringes and collected in plain and EDTA bulb (Ethylene diamine tetraacetic acid) 1ml in each bulb and immediately transferred to the laboratory for analysis.

**Estimation of CRP**

The CRP-Turbilatex is a quantitative turbidimetric test measurement of C-reactive protein (CRP) in human serum or plasma. Latex particles coated with specific human anti-CRP are agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance change, dependent upon the CRP contents of the patient sample that can be quantified by comparisons from a calibrator of known CRP concentration.

**Estimation of WBC count, RBC count, hemoglobin count**

CBC Cell Counter was used for estimation of WBC Count (Neutrophil, Lymphocyte), RBC Count and Hemoglobin count. Digitally printed results were obtained. Peripheral smear was used for Eosinophil and Monocyte count.

**Statistical analysis**

To test the equality of means of the three test groups and control group, ANOVA (Analysis of Variance) was carried out for each parameter. Multiple comparisons made by comparing differences between the means within the groups, were evaluated by applying Scheff’s analysis. Statistical analysis carried out by using statistical software SPSS® version 8.0. The p value evaluated at the 5% level of significance.

**Results**

Table 1 shows comparison between means of (levels of CRP concentration) of group A, B, C and control group. The highest mean concentration for CRP (6.0033) was obtained for group ‘C’ and the least mean concentration (1.9800) was obtained for control group. The mean concentration for group ‘A’ (3.6013) was intermediate between the control group and group ‘B’ (5.8300). Mean for group ‘B’ was intermediate between group ‘A’ and group ‘C’. The mean obtained for group A, B, C, and control group were (3.6013, 5.8300, 6.0033, and 1.9800) respectively, with a P value = 0.040 (< 0.05). Similarly, the mean obtained for group A, B, C, and control group were (3.6013, 5.8300, 6.0033, and 1.9800) respectively, with a P value = 0.040, the four means differs significantly according to ANOVA test.

Table 2 shows multiple comparisons between CRP levels amongst all the groups. These results suggested that level of CRP concentration in serum remained high in group ‘C’ and least concentration in control group. The mean for control group was least and highest in group ‘C’. These results showed that levels of CRP concentration increases progressively as the disease progress from control group to group ‘C’, with highest concentration in group ‘C’.

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### Table 1: Comparison between means of (levels of CRP concentration) of group A, B, C and control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>F Value</th>
<th>P Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
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<td>3.0800</td>
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<td>2.866</td>
<td>0.040</td>
<td>Sig</td>
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<td>Mild</td>
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<td>5.8300</td>
<td>8.1845</td>
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<td></td>
</tr>
<tr>
<td>Severe</td>
<td>30</td>
<td>6.0033</td>
<td>9.0640</td>
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</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>4.3537</td>
<td>6.3680</td>
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<td></td>
</tr>
</tbody>
</table>

### Table 2: Multiple comparisons between means of (levels of CRP concentration) of group A, B, C and control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group</th>
<th>Mean Difference</th>
<th>P Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
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<td>Control</td>
<td>Mild</td>
<td>-1.6213</td>
<td>.797</td>
<td>NS</td>
</tr>
<tr>
<td>Control</td>
<td>Moderate</td>
<td>-3.8500</td>
<td>.131</td>
<td>NS</td>
</tr>
<tr>
<td>Control</td>
<td>Severe</td>
<td>-4.0233</td>
<td>.105</td>
<td>NS</td>
</tr>
<tr>
<td>Mild</td>
<td>Moderate</td>
<td>-2.2287</td>
<td>.590</td>
<td>NS</td>
</tr>
<tr>
<td>Mild</td>
<td>Severe</td>
<td>-2.4020</td>
<td>.527</td>
<td>NS</td>
</tr>
<tr>
<td>Moderate</td>
<td>Severe</td>
<td>-1.733</td>
<td>1.000</td>
<td>NS</td>
</tr>
</tbody>
</table>

### Table 3: Comparison between means of (levels of WBC count) of group A, B, C and control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>F Value</th>
<th>P Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>6116.6667</td>
<td>1759.1305</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>30</td>
<td>6890.0000</td>
<td>1174.5432</td>
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<td></td>
</tr>
<tr>
<td>Moderate</td>
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<td>7290.0000</td>
<td>2789.4320</td>
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<td></td>
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<tr>
<td>Severe</td>
<td>30</td>
<td>7646.6667</td>
<td>2124.8502</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>6985.8333</td>
<td>2100.7120</td>
<td>3.086</td>
<td>0.030</td>
<td>Sig</td>
</tr>
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</table>

### Table 4: Multiple comparisons between means of (levels of WBC count) of group A, B, C and control group.

The mean obtained for group A, B, C, and control group were (6890.0000, 7290.0000, 7646.6667, and 6116.6667) respectively, with a P value = 0.030 (< 0.05). Similarly, the mean obtained for group A, B, C and control group were (6890.0000, 7290.0000, 7646.6667 and 6116.6667) respectively, with a P value = 0.030, the four means differs significantly according to ANOVA test.

As the means were not equal, a pair wise comparison of means was carried out using Scheff’s Analysis. Control was compared with group ‘A’ (P value = 0.546 > 0.05). Control group was compared with group ‘B’ (P value = 0.184 > 0.05). Control group was compared with group ‘C’ (P value = 0.044 < 0.05). Group ‘A’ was compared with group ‘B’ (P value = 0.902 > 0.05). Group ‘A’ was compared with group ‘C’ (P value = 0.564 > 0.05). Group ‘B’ was compared with group ‘C’ (P value = 0.928 > 0.05). Table 4 shows multiple comparisons between means of (levels of WBC count) of group A, B, C and control group. These results suggested that level of WBC count in serum remains

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high in group ‘C’ and least count in control group. These results showed that levels of WBC count increases progressively as the disease progress from control group to group ‘C’, with highest count in group ‘C’, and significant difference was found between mean of control group and group ‘C’.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group</th>
<th>Mean Difference</th>
<th>P Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
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<td>Mild</td>
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<td>.546</td>
<td>NS</td>
</tr>
<tr>
<td>Control</td>
<td>Moderate</td>
<td>-1173.3333</td>
<td>.184</td>
<td>NS</td>
</tr>
<tr>
<td>Control</td>
<td>Severe</td>
<td>-1530.0000</td>
<td>.044</td>
<td>Sig</td>
</tr>
<tr>
<td>Mild</td>
<td>Moderate</td>
<td>-400.0000</td>
<td>.902</td>
<td>NS</td>
</tr>
<tr>
<td>Mild</td>
<td>Severe</td>
<td>-756.6667</td>
<td>.564</td>
<td>NS</td>
</tr>
<tr>
<td>Moderate</td>
<td>Severe</td>
<td>-356.6667</td>
<td>.928</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Table 4: Multiple comparisons between means of (levels of WBC count) of group A, B, C and control group.*

The highest mean count for RBC (4763333.3333) was obtained for control group and the least mean count (4226000.0000) was obtained for group ‘C’. The mean count for group ‘A’ (4414000.0000) was intermediate between group ‘B’ (4326666.6667) and control group. Mean for group ‘B’ was intermediate between group ‘C’ and group ‘A’. Table 5 shows comparison between means of (levels of RBC count) of group A, B, C and control group. The mean obtained for group A, B, C and control group were (4414000.0000, 4326666.6667, 4226000.0000, and 4763333.3333) respectively, with a P value = 0.030 (< 0.05). So, the hypothesis of means was rejected at 5% level of significance (P value = 0.020 < 0.05).

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>F Value</th>
<th>P Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>4763333.3333</td>
<td>749387.4894</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>30</td>
<td>4414000.0000</td>
<td>507010.1679</td>
<td>3.421</td>
<td>0.020</td>
<td>Sig</td>
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<td>Moderate</td>
<td>30</td>
<td>4326666.6667</td>
<td>775203.3474</td>
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<tr>
<td>Severe</td>
<td>30</td>
<td>4226000.0000</td>
<td>702790.4971</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>4432500.0000</td>
<td>712437.2300</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 5: Comparison between means of (levels of RBC count) of group A, B, C and control group.*

Similarly, the mean obtained for group A, B, C and control group were (4414000.0000, 4326666.6667, 4226000.0000, and 4763333.3333) respectively, with a P value = 0.020, the four means differs significantly according to ANOVA test.

As the means were not equal, a pair wise comparison of means was carried out using Scheff’s Analysis. When Control group was compared with group ‘A’ P value was found to be 0.286 > 0.05. Control group was compared with group ‘B’ (P value = 0.119 > 0.05). Control group was compared with group ‘C’ (P value = 0.033 < 0.05). Group ‘A’ was compared with group ‘B’ (P value = 0.971 >0.05). Group ‘A’ was compared with group ‘C’ (P value = 0.775 > 0.05). Group ‘B’ was compared with group ‘C’ (P value = 0.956 > 0.05). Table 6 depicts multiple comparisons between means of (levels of RBC count) of group A, B, C and control group.

After Scheff’s Analysis significant difference was found between mean of control group and group ‘C’. The levels of RBC count in serum were found to be high in control group, and least count in group ‘C’.

These results showed that, mean for control group was highest in control group and least in group ‘C’. These results showed that levels of RBC count decreased progressively as the disease progresses from control group to group ‘C’, with highest count in control group, and significant difference was found between mean of control group and group ‘C’.

Table 7 shows comparison between means of (levels of Hb% count) of group A, B, C and control group. The highest mean concentration for Hb (11.8833) was obtained for control group and the least mean concentration (10.7100) was obtained for group ‘C’. The mean concentration for group ‘A’ (11.0633) was intermediate between group ‘B’ (11.1167) and control group. Similarly, the mean obtained for group A, B, C, and control group were (11.0633, 11.1167, 10.7100, and 11.8833) respectively, with a P value = 0.041, the four means differs significantly according to ANOVA test.

Table 8: Multiple comparisons between means of (levels of Hb% count) of group A, B, C and control group.

As the means were not equal, a pair wise comparison of means was carried out using Scheff’s Analysis (Table 8). Control was compared with group ‘A’ (P value = 0.276 > 0.05). Control group was compared with group ‘B’ (P value = 0.336 > 0.05). Control group was compared with group ‘C’ (P value = 0.051 > 0.05). Group ‘A’ was compared with group ‘B’ (P value = 0.999 > 0.05). Group ‘A’ was compared with group ‘C’ (P value = 0.867 > 0.05). Group ‘B’ was compared with group ‘C’ (P value = 0.810 > 0.05).
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Discussion

Literature suggests that elevated peripheral blood levels of several systemic inflammatory markers including CRP, fibrinogen, and the cytokines; IL-1β and IL-6 and tumor necrosis factor alpha (TNF-α), are associated with risk of cardiovascular diseases and the severity of atherosclerosis. CRP, named for its capacity to precipitate the somatic C-polysaccharide of Streptococcus pneumoniae, was the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage [20].

The acute-phase response comprises the nonspecific physiological and biochemical responses of endothermic animals to most forms of tissue damage, infection, inflammation, and malignant neoplasia. In particular, the synthesis of a number of proteins is rapidly upregulated, principally in hepatocytes, under the control of cytokines originating at the site of pathology.

These results suggested that level of CRP concentration in serum remained high in group ‘C’ and least concentration in control group. These results showed that, mean for control group was least and highest in group ‘C’. These results showed that levels of CRP concentration increases progressively as the disease progress from control group to group ‘C’, with highest concentration in group ‘C’. The results of this study confirm the outcomes of studies which reported an elevation of the CRP levels in periodontitis patients. Studies by Noack, et al. [18] and Slade, et al. [21] reported increasing levels of CRP concentration with increasing disease severity. Slade, et al. reported that dentate people with extensive periodontal disease (> 10% of sites with periodontal pockets 4+ mm) had an increase of approximately one-third in mean CRP and a doubling in prevalence of elevated CRP compared with periodontally healthy people. Raised CRP levels among people with extensive periodontal disease persisted in multivariate analyses (P < 0.01), even after controlling for established risk factors for elevated CRP (diabetes, arthritis, emphysema, smoking, and anti-inflammatory medications) and socio-demographic factors.

Noack, et al. (2001) also reported significantly higher mean CRP levels (4.06 ± 5.55 mg/l) with high levels of mean attachment levels, than controls [(1.70 ± 1.91mg/l), P = 0.011]. The results of this study are in full agreement with Noack, et al. [18] and Slade, et al [8].

Several underlying mechanism for this observation are possible. Periodontal pathogens do not induce only local inflammation and tissue destruction, Systemic manifestation of this disease is also detected to many oral bacteria and appears to be increased with periodontitis, potentially resulting from transient access of oral bacteria to the circulation.

They are involved in systemic increases in inflammatory and immune responses. Low levels of bacteremia, LPS, and other bacterial components may provide a stimulus for systemic inflammatory responses such as increased production of CRP due to activating the cascade of inflammatory cytokine production by monocytes and other cells in periodontal tissue and elsewhere. These results suggested that level of WBC count in serum remains high in group ‘C’ and least count in control group.

The results of the present study showed that, mean for control group was least and highest in group ‘C’. These results showed that levels of WBC count increases progressively as the disease progress from control group to group ‘C’, with highest count in group ‘C’, and significant difference was found between mean of control group and group ‘C’. Kweider, et al. [22] reported that periodontitis patient had significantly higher levels of WBC count in periodontitis patients compared to control subjects.

The cause for anemia of chronic disease is most likely multi-factorial however it is currently thought that pro-inflammatory cytokines from a given chronic disease process such as rheumatoid arthritis, may down regulate the erythropoiesis in bone marrow [24]. In particular interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor- alpha (TNF-α), have been implicated as cytokines responsible suppressing erythropoiesis [25,26]. The levels of Hb concentration in serum was found to be high in control group, and least concentration in group ‘C’. Hutter, et al. [23] has reported lower number of red blood cells and hemoglobin count in periodontitis patients, (moderate and severe) as compared to control subjects (P = 0.001, P < 0.001, P = 0.002) respectively. The results of this study are in agreement with Hutter, et al. study. The current finding indicates that periodontitis also needs to considered as a chronic disease which may cause lower numbers of erythrocytes and consequently lower hemoglobin levels.

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The pathogenesis of anemia in periodontitis patients is most likely due to depressed erythropoiesis by systemically circulating cytokines resulting from a local chronic inflammatory process. Thus, it can be inferred from the findings of the present study that as the severity of chronic periodontitis increases the chances of developing anemia increases thereby again leading to disruption of cardiovascular events. As literature reveals that anemia is related to chronic heart failure it may be considered as one of the mechanism that relates periodontitis to cardiovascular diseases.

Pre assessment of serum C reactive protein and complete blood count in chronic periodontitis can predict the risk of developing cardiovascular disease and thereby can help in diagnosis at earlier stages and can aid in providing screening services and advice to seek immediate dental care.

Conclusion
The serum levels of CRP increases with the severity of periodontal disease, thereby increasing the risk of Cardiovascular disease. The WBC count also was found to be increased in severe chronic periodontitis cases thereby suggesting active inflammation. Whereas the RBC count and Hb count significantly decreases with the severity of periodontitis thereby increasing risk of anemia. These conclusions stress that link between periodontal disease and cardiovascular diseases. It also shed light on possible mechanism that links these two diseases. As periodontitis is a treatable condition, appropriate interventions to reduce or prevent periodontal disease should be undertaken. This may reduce the risk of cardiovascular disease at least to some extent.

Bibliography


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