Original Article

Estimation and Comparison of Erythrocyte and Hemoglobin Levels in Subjects with Healthy Periodontium and Chronic Periodontitis

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Abstract

Objective: To compare and evaluate the relationship between anemia and periodontitis by estimation of peripheral blood between healthy patients and chronic periodontitis patients. Material and Methods: Of the total of 230 outpatients approached to participate in the study, 100 eligible patients were selected as per the selection criteria. After written consent, these patients were divided into two groups according to the clinical parameters as healthy and disease (chronic periodontitis) groups. Under aseptic conditions, venous blood samples were obtained by vein-puncture in the ante-cubital fossa without excessive venous stasis and the mean value of erythrocytes (EC), hemoglobin concentration (HGB), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were measured. Paired t-test was employed to find the significance of study parameters on continuous scale within the group analysis and unpaired t-test (two tailed, dependent) was used to find the significance of study parameters on continuous scale in the inter group analysis. Results: Generally, the healthy group reported higher levels of EC, HGB, MCV and MCH than the periodontitis group. The mean hemoglobin level was significantly higher (p-value<0.05) in healthy patients (12.66 ±1.41 gm/dl) whereas a slightly lower level of 11.32±1.85 gm/dl was observed in patients with chronic periodontitis. Similarly, the range of erythrocyte count of healthy patients was significantly higher (3.69-5.29 million/µl) than chronic periodontitis patients (3.33-5.97 million/µl). Whilst MCV was higher in healthy patients but non-significant, higher mean MCH of 27.75 ± 3.25 pg/cell was reported in healthy patients compared to mean of 25.73 ± 3.22 pg/cell in patients with chronic periodontitis. Conclusion: Significant hematological differences in EC, HGB, MCV and MCH between healthy periodontium and chronic periodontitis subjects were seen indicating mild anemia.

Keywords: Periodontitis; Anemia; Oral Health; Hemoglobins.
Introduction

Periodontium is a complex and highly specialized pressure sensing system consisting of four components such as cementum, periodontal ligament, alveolar bone, junctional and sulcular epithelia supporting the teeth. Of these structures, periodontal ligament is a dynamic tissue with a high rate of remodeling and turnover, which connects the teeth to the alveolar bone [1]. However, microbial substances gain access to the gingival tissue and initiate and perpetuate an inflammatory reaction, which leads to the destruction of the periodontal ligament and alveolar bone, leading to chronic periodontitis and, finally, to tooth loss if left untreated [2]. In the industrialized countries, moderate or severe forms of destructive periodontal disease affect almost 40% of the adult population [3,4]. Periodontitis has even higher prevalence in developing countries and considerable global variation, although the prevalence of severe generalized disease appears to be similar in most populations [5].

Periodontitis in itself, is a chronic infectious disease of the supporting tissues of the teeth, with multiple related factors [6]. The most common etiology of periodontal disease is dental plaque, which consists of microbial flora containing more than 700 distinct microbial species cultivated from dental plaque [7]. The clinical symptoms of this disease include swollen red gingiva, gingival bleeding, suppuration, periodontal pocketing, gingival recession and loss of supporting alveolar bone.

With the onset of the above symptoms, prevention of entry of microorganisms and other irritants in the protective barrier system of sulcular epithelium and junctional epithelium is lost. Periodontal pathogens can, thus, disseminate through the blood and can contribute to development of adverse systemic effects by direct (endothelial injury or dysfunction) indirect (through lipopolysaccharide (LPS) activated cytokine profiles) mechanisms [8]. This host-microbial interaction in periodontitis leads to ulceration of pocket epithelium around affected teeth and forms a porte d’entre´ for bacteria and their products, such as endotoxin [9,10]. Bacteraemia in periodontitis has been demonstrated and the extent is directly related to the severity of the inflammation of the periodontal tissues [9,10].

In view of above, some authors concluded that like any other chronic condition, chronic periodontitis can lead to anemia [11]. In India, it is a common and serious health disorder among both the genders and all age groups, with a higher prevalence among females [12]. Anemia of chronic disease (ACD) is the second most prevalent anemia after iron deficiency anemia (IDA) and occurs in patients with acute or chronic immune activation. Thus, the condition has been termed "anemia of inflammation" [12]. ACD is reported to be one of the most common forms of anemia observed in clinical medicine [13-15]. Typically, it is a mild to moderate, normochromic / normocytic anemia and is characterized by decreased serum iron and total iron binding capacity, with normal or increased iron stores and develops after a month or two of active disease [15].

Different enzymes are released by stromal, epithelial or inflammatory cells which reflect metabolic changes in the gingival and periodontium in inflammation [12]. It is currently thought that pro-inflammatory cytokines, macrophage-derived most notably tumor necrosis factor -alpha
(TNF-α), transforming growth factor-beta (TGF-β), interleukin-1 alpha (IL-1α), interleukin-beta (IL-β) and interleukin-6 (IL-6) from a given chronic disease process may down-regulate erythropoiesis and suppress the bone marrow response to erythropoietin, thus possibly contributing to anemia [12,16].

Thus, initiation and progression of gingivitis and periodontitis may be affected by certain systemic conditions. This means that there may be potential effects of periodontal disease on a wide range of organ systems. As the periodontal tissues mount an immune inflammatory response to bacteria and their products, systemic challenges with these agents also induce a major vascular response offering explanatory mechanisms for the interaction between periodontal infections and a variety of systemic disorders [17,18]. Epidemiological studies have suggested that periodontal deterioration increases the risk of systemic problems such as chronic obstructive pulmonary diseases (COPDs), stroke [19], cardiovascular diseases (CVDs) [20], atherosclerosis, diabetes mellitus (DM) [21], adverse pregnancy outcomes [22]. Periodontal diseases have proved to be an independent risk factor for adverse pregnancy disorders such as preterm births, low birth weight and pre-eclampsia, after adjusting for other confounding factors [23].

These associations, thus, suggest that periodontal diseases may have systemic effects. In addition, some studies have found that periodontal infection elicit systemic blood chemistry changes [24]. It has therefore been speculated that periodontitis results in a low-grade systemic inflammation, which may cause lower number of erythrocytes and consequently, lower hemoglobin concentration [25,26]. Recently it has been demonstrated that periodontitis is associated with elevated numbers of white blood cells (WBCs) and elevated levels of C-reactive protein (CRP) [27-29]. In view of above, there has been renewed interest to study the association of periodontitis and changes in cellular and molecular components of peripheral blood.

Therefore, the present study aims to compare and evaluate the relationship between anemia and periodontitis by estimation of peripheral blood in relation to the erythrocyte count (EC), hemoglobin (HGB) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) in healthy controls with normal periodontium and chronic generalized periodontitis patients.

**Material and Methods**

**Sample Characteristics**

The study was conducted at the department of Periodontology and Oral Implantology, Maharaja Ganga Singh Dental College and Research Center with the approval of institutional ethical committee. A total of 230 patients were approached of which 100 eligible patients were randomly selected from the outpatient department of the college. Prior to the study, the design and purpose of the study was verbally explained to all the patients. A detailed Performa was prepared about the purposes of the investigation and a written consent was procured from them before treatment.

**Criteria for Selection of Patients**
Male and female patients between 30-55 years of age were randomly selected from the outpatient list. Patients having at least 20 remaining natural teeth with probing depths more than 5 mm were selected for this study. Patients who were medically compromised and those with history of medications and vitamin supplements in the last three months were excluded from the study. Furthermore, patients who have had tooth extractions or undergone periodontal treatment in the last six weeks were excluded from participation. Past and present smokers and alcoholics as well as pregnant or lactating women were also excluded from the study.

Data Collection

A detailed case history was procured from all the patients and the record of the patients was maintained as per the attached Performa to have a systematic and methodical recording of all the information and observations. The intraoral clinical examination was done in a dental chair, under standard conditions of light, using a mouth mirror, explorer, graduated periodontal probe and tweezers. Clinical parameters such as gingival and plaque index, periodontal probing depth and clinical attachment levels were recorded by visual examination on the four surfaces of the tooth for each patient by a single examiner.

Based upon the clinical parameters measured, the subjects to be evaluated were divided into two groups as Group 1 with 50 subjects with healthy periodontium and Group 2 with 50 subjects with chronic periodontitis.

Hematological Investigations

Under aseptic conditions, venous blood samples were obtained by vein-puncture in the antecubital fossa without excessive venous stasis. The blood was taken into ethylenediamine tetra-acetic acid (EDTA) containing vacuum tubes [Trade International K3 EDTA anticoagulant test tube (2ml)] and transported to a clinical pathology for processing within 4 hours after vein-puncture. In standardized and automated procedures, the mean value of erythrocytes, hemoglobin concentration, MCV and MCH were measured on a Cellydyn 3500 (Vector Mindray complete blood count machine).

Statistical Analyses

The descriptive statistical analysis was carried out in the present study. Results on continuous measurements were presented on Mean ± SD (Min-Max). Paired t-test was employed to find the significance of study parameters on continuous scale within the group analysis and unpaired t-test (two tailed, dependent) was used to find the significance of study parameters on continuous scale in the inter group analysis.

Ethical Aspects
Ethical approval for this dental public health survey was obtained from the Ethical Committee of Maharaja Ganga Singh Dental College and Research Centre.

Results

Of the total of 230 patients, 100 patients who fulfilled the inclusion criteria were randomly selected from the pool of outpatients and were divided into healthy (Group 1) and chronic periodontitis (Group 2) based on the clinical parameters. The mean age in Group 1 was 34.92 years and in group 2, 45.72 years as seen in Table 1. In terms of gender distribution, 60% of the sample were males and 40% were females.

Table 1. Age distribution of the groups with mean and standard deviation

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>'t' value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (Healthy)</td>
<td>50</td>
<td>30 - 53</td>
<td>34.92 ± 5.68</td>
<td>8.873</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>G2 (Chronic Periodontitis)</td>
<td>50</td>
<td>32 - 55</td>
<td>45.72 ± 6.47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Statistically significant; SD: Standard deviation.

The scores of the hematological investigations were statistically analyzed by calculating the mean values and standard deviation as seen in Table 2. Generally, the healthy group reported higher levels of mean hemoglobin, Mean erythrocyte, MCV and MCH than the periodontitis group. For mean hemoglobin levels, higher values were seen in healthy patients (Group 1) compared to patients with chronic periodontitis (Group 2). The mean hemoglobin level was significantly higher (p-value<0.05) in healthy patients (12.66 ±1.41 gm/dl) (Group 1) whereas a slightly lower level of 11.32±1.85 gm/dl was observed in patients with chronic periodontitis (Group 2). Similarly, the range of erythrocyte count of healthy patients was significantly higher (3.69-5.29 million/µl) than chronic periodontitis patients (3.33-5.97 million/µl).

The mean MCV was 88.73 ± 9.81 fm/cell in healthy patients (Group 1) compared to 85.68 ± 8.39 fm/cell seen in patients with chronic periodontitis (Group 2) but was non-statistically significant. Finally, the mean MCH of healthy patients was 27.75 ± 3.25 pg/cell whereas it was 25.73 ± 3.22 pg/cell in patients with chronic periodontitis.

Table 2. t-test of hematological investigations with range, mean, standard deviation and p-values.

<table>
<thead>
<tr>
<th>Hematological Investigations</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>'t' value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Hemoglobin Level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1 (Healthy)</td>
<td>9.50 - 15.00</td>
<td>12.66 ± 1.41</td>
<td>4.077</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>G2 (Chronic Periodontitis)</td>
<td>6.50 - 15.60</td>
<td>11.32 ± 1.85</td>
<td></td>
<td></td>
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<tr>
<td>Mean Erythrocyte Level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1 (Healthy)</td>
<td>3.69 - 5.29</td>
<td>4.57 ± 0.41</td>
<td>1.991</td>
<td>0.049**</td>
</tr>
<tr>
<td>G2 (Chronic Periodontitis)</td>
<td>3.33 - 5.97</td>
<td>4.40 ± 0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Corpuscular Volume Level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1 (Healthy)</td>
<td>55.80 - 116.10</td>
<td>88.73 ± 9.81</td>
<td>1.671</td>
<td>0.098NS</td>
</tr>
<tr>
<td>G2 (Chronic Periodontitis)</td>
<td>62.20 - 101.30</td>
<td>85.68 ± 8.39</td>
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<tr>
<td>Mean Corpuscular Hemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1 (Healthy)</td>
<td>21.20 - 37.40</td>
<td>27.75 ± 3.25</td>
<td>3.121</td>
<td>0.002*</td>
</tr>
<tr>
<td>G2 (Chronic Periodontitis)</td>
<td>17.20 - 30.10</td>
<td>25.73 ± 3.22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Statistically significant; NS: Non-significant; SD: Standard deviation.
Discussion

The present study aims to compare and evaluate the relationship between anemia and periodontitis by estimation of peripheral blood between healthy patients and chronic periodontitis patients. In this study, the mean age of cases of healthy periodontium (Group 1) was 34.92 ± 5.68 whereas in chronic periodontitis (Group 2) the mean age was 45.72 ± 6.47. This fact correlates with the similar studies [30,31], which indicate that the prevalence of periodontal disease increases concomitantly with age.

The data analysis in this study showed a lower number of EC in periodontitis patients (4.57 ± 0.41) as compared to healthy subjects (4.40 ± 0.47), which was in accordance to other studies [32-34]. It can be seen from the above studies that periodontitis patients have elevated levels of acute phase proteins, IL-1, IL-6 and TNF-α which suppress mature erythroid progenitors and inhibit in-vitro colony formation by erythroid burst-forming units and erythroid colony forming unit leading to decrease in EC.

Various studies have tried to evaluate the relationship between periodontitis and HGB levels. The current study suggests that periodontitis also needs to be considered as a chronic disease, which may cause a lower number of EC, and subsequently lower amount of HGB levels suggestive of anemia of chronic disease in a substantial number of patients. A previous also showed a positive relation between decrease of EC and progression of periodontal disease [32]. In addition, the results substantiate the findings put forth by some authors [29,35], who also reported decrease in the number of erythrocytes, which was likely to be secondary to the presence of periodontal disease, since the arrest or cure of these periodontal conditions resulted in elevation of EC to normal. Subsequently, another authors found an increased in HGB and RBC levels in patients with severe periodontitis after scaling and root planning [36].

The difference of MCV between healthy subjects and chronic periodontitis patients was statistically insignificant (p= 0.098) as the mean ± SD was 88.73 ± 9.81 and 85.68 ± 8.39 respectively. Similar results in MCV were seen in other studies [37-39]. A decrease in MCH value is seen in microcytic anemia caused due to iron deficiency, whereas an increase in MCH value is seen in macrocytic anemia caused by vitamin deficiency. However, in this study the MCH values were lower in chronic periodontitis patients as compared to healthy subjects but the values of both the group were within the normal range of MCH indicating the anemia to be normocytic as seen in ACD as reported previously [40].

It was observed that a lower HGB level and EC in periodontitis patients was seen as compared to controls which is suggestive of anemia whereas the MCV values were in the normal reference range for both chronic periodontitis patients and periodontally healthy patients which suggested that the anemic status was not due to iron deficiency or vitamin deficiency but normocytic anemia. The IDA is associated with decreased MCV values (microcytosis) whereas vitamin deficiency is associated with elevated levels of MCV (macrocytosis). As the MCV values were in the normal range in the present study, it indicates normocytosis suggestive of anemia of chronic disease.
Only few reports in the literature have investigated the bi-directional relationship between anemia and periodontitis. Most of the authors believe that anemia was one of the causes of periodontitis rather than being the consequences. Thus, the hematological parameters like EC, HGB, MCV and MCH were evaluated in the present study, as these are indicative of anemic state of the patient and also the type of anemia based on the morphology of the cell. Anemia has not been identified in the recent literature as a systemic consequence of periodontitis. The pathogenesis for the current findings is most likely similar as reported for RA, that is depressed erythropoiesis by systemically circulating pro inflammatory cytokines resulting from a local chronic inflammatory process. In support of this concept are the observed systemic levels of IL-6 in about 1/3 of patients with localized periodontitis and in about half of patients with generalized periodontitis [41,42].

The findings of this study suggest the understanding that chronic infections such as periodontitis have systemic effects in terms of blood parameters, indicating anemia. However, there are limitations in this study, which could be improved, in further investigatory studies. In the present study, the periodontal conditions were not intervened by way of treatment and measurements later on, hence, it was not possible to comment whether anemia was a factor that caused periodontitis or a consequence. The blood parameters of chronic periodontitis patients were not available in their healthy state, and so, it becomes difficult to ascertain whether the changes in blood parameters occurred due to the diseased condition. A comparison of post-therapeutic blood parameters in patients suffering from chronic periodontitis would add to our understanding with regard to the changes in blood parameters in chronic periodontitis. Analysis of serum ferritin levels and soluble serum transferring receptor concentration or a bone marrow examination is necessary to quantify the iron stores and definitely distinguish between anemia of chronic disease and iron deficiency anemia.

**Conclusion**

Significant hematological differences in erythrocyte count, hemoglobin levels, mean corpuscular volume and mean corpuscular hemoglobin between healthy periodontium and chronic periodontitis subjects were seen indicating mild anemia. Thus, subjects with periodontitis can be considered to be at risk for mild anemia of chronic disease. Therefore, it be concluded that chronic periodontitis as a possible contributing factor or a cause of mild anemia as indicated in this study.

**References**


