



## Comparative evaluation of hematological parameters in periodontally healthy, gingivitis and periodontitis subjects

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### ABSTRACT

Anemia of chronic disease (ACD) is defined as anemia occurring in chronic infections, inflammatory conditions or neoplastic disorders that are not due to marrow deficiencies or other diseases, and occurring despite presence of adequate iron stores and vitamins. The main underlying reason for ACD is previous chronic inflammation or infection. The present study was conducted to evaluate and compare the hematological parameters in periodontally healthy, gingivitis and periodontitis subjects and thereby assess the relationship between anemia and periodontitis. A total of 60 subjects of age group between 20 and 55 years were recruited as periodontally healthy (n=20), gingivitis (n=20), periodontitis (n=20) groups based on their CPI scores. The hematological parameters: Red blood cells (RBC), Hemoglobin (Hb), Erythrocyte sedimentation rate (ESR) were recorded for all the three groups. Statistical test used was ANOVA. The mean RBC score and Hb score were found to be highest in healthy group, followed by gingivitis group and periodontitis group, whereas the mean ESR score was found to be highest in periodontitis group, followed by gingivitis group and healthy group. It was concluded from the study that a positive relationship was observed between hematological parameters and severity of periodontal disease, suggesting that like any other chronic disease periodontitis may also leads to anemia.

**Key words:** Anemia, Gingivitis, Periodontitis, Red blood cells, Hemoglobin

### INTRODUCTION

Periodontitis is an infectious disease that causes destruction of the tooth attachment apparatus [1] which is characterized by the presence of gingival inflammation, periodontal pocket formation, and loss of connective tissue attachment and alveolar bone around the affected teeth. [2] The initiation and progression of gingivitis and periodontitis may be affected by certain systemic conditions. The converse side of the relationship between systemic health and oral health has also been demonstrated. This means that there may be potential effects of periodontal disease on a wide range of organ systems. [3] Epidemiologic studies suggest that periodontal problems increase the risk of systemic problems like cardiovascular diseases, cerebrovascular diseases, atherosclerosis, preterm low birth weight, and diabetes mellitus. [4] The association of periodontal disease with several systemic conditions such as diabetes and atherosclerotic disease is reported to be related to the inflammatory response of periodontal disease. C-reactive protein is an important marker of the inflammatory response and is elevated in subjects with periodontal disease; Its levels in peripheral blood are reduced when periodontal disease is treated. Another indication of the systemic inflammatory response associated with periodontal disease is the elevated levels of cytokines, including tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukins 1 and 6 (IL-1,6) often found in the circulation of patients with periodontal disease. [5] This suggests a possible influence of periodontitis on systemic status of an individual.

Anemia is defined as a state of reduced Hb concentration, reduced number of circulating erythrocytes in the blood, or both. In the literature, early studies [6-8] reported anemia in periodontitis. Anemia of chronic disease (ACD) is defined as anemia occurring in chronic infections, inflammatory conditions or neoplastic disorders that are not due to marrow deficiencies or other diseases, and occurring despite presence of adequate iron stores and vitamins. [9] It is characterized by blunted erythropoietin response by erythroid precursors, decreased red blood cell survival and a defect in iron absorption and macrophage iron retention, which interrupts iron delivery to erythroid precursor cells. The main underlying reason for ACD is previous chronic inflammation or infection. According to previous studies, increase of some inflammatory response cytokines such as TNF- $\alpha$ , interferon and IL-1 is observed in ACD. [17] The whole process of the ACD can relate to these cytokines which leads to decreased RBC life span, and impaired development of erythroid. In addition, it decreases erythropoietin response to anemia. Significant increase in TNF- $\alpha$ , IL-6, and IL-1 $\beta$  was observed in patients suffering from ACD suggesting the role of these cytokines in preventing erythroid maturation and differentiation. [10-12] Because of similar cytokines in pathogenesis of ACD and periodontitis, and also as periodontitis is a chronic infection, a relationship between these two diseases was proposed. Several studies have been conducted by various authors to assess the hematological parameters in periodontitis subjects. Sneha R Gokhale *et al* in 2010 have evaluated blood parameters like hematocrit, number of erythrocytes and hemoglobin levels between periodontally healthy controls and diseased individuals. [13] Also Shobha Prakash *et al* in 2012 has evaluated and compared the levels of hemoglobin, hematocrit, ESR, serum iron and ferritin levels between subjects with and without periodontitis. [15] No studies till now have evaluated the hematological parameters in gingivitis group along with healthy and periodontitis group. Hence the aim of the present study is to evaluate and compare the hematological parameters in periodontally healthy, gingivitis and periodontitis subjects.

## EXPERIMENTAL SECTION

### Materials and methods

In the present cross sectional study, a total of 60 subjects were recruited as periodontally healthy (n=20), gingivitis (n=20), and periodontitis (n=20) from the out patients visiting the department of periodontology, D.A.P.M .R.V Dental college, Bangalore. Ethical clearance was obtained for the study and all subjects signed informed consent documents for participation in the study.

### Inclusion criteria

- Both male and female patients of age between 20 and 55 years
- No systemic diseases or acute or chronic medical conditions including diabetes, viral, fungal or bacterial infections.
- No history of periodontal therapy prior to 6 months of examination.
- Periodontal parameters showing signs of health, gingivitis or periodontitis

### Exclusion criteria

- Pregnant and lactating patients.
- Smokers
- Patients with history of hospitalization or intake of medications in the last 6 months.
- Patients with a known history of any bleeding disorders.

### Clinical assessment

A complete periodontal examination was undertaken using a mouth mirror and CPITN probe. Subjects were categorized as periodontally healthy, gingivitis and periodontitis based on CPI scores. CPI score 0 – Healthy, CPI score 1, 2- Gingivitis, CPI score 3, 4 – Periodontitis.

### Hematological assessment

Under aseptic measures, venous blood samples were drawn by venipuncture in antecubital fossa using a 5-ml syringe, collected into EDTA-containing vacuum tubes, and transported to a clinical laboratory for RBC analyses <3 hours after collection. Hb%, number of erythrocytes (RBC), Erythrocyte Sedimentation Rate (ESR) was estimated. The erythrocyte sedimentation rate (ESR) was measured according to Westergren procedures.

### Statistical test used is ANOVA.

Intergroup comparisons were carried out using Bonferroni test.

## RESULTS AND DISCUSSION

The results of the study are as follows:

**Mean RBC among the groups:**

The mean RBC among all the three groups has been shown in Table 1 and Graph 1. In healthy group the mean RBC scores were  $6.415 \pm 9.045$ . In gingivitis group the mean RBC scores were  $4.605 \pm 0.516$ . In periodontitis group the mean RBC scores were  $4.865 \pm 4.865$ . The mean RBC score was found to be highest in healthy group, followed by periodontitis group and gingivitis group respectively. However, the difference in mean RBC score among the three groups were not statistically significant ( $P > 0.05$ ).

TABLE 1: Mean RBC values in different groups

Group	Mean	Std Dev	SE of Mean	95% CI for Mean		Min	Max
				Lower Bound	Upper Bound		
Healthy	6.415	9.045	2.023	2.182	10.648	3.6	44.8
Gingivitis	4.605	0.516	0.115	4.364	4.846	3.8	5.2
Periodontitis	4.865	4.865	0.135	4.583	5.147	3.9	5.7

TABLE 2: Comparison of mean RBC values between the groups:

Group I	Group J	Mean Difference (I-J)	P-Value	95% CI	
				Lower Bound	Upper Bound
Healthy	Gingivitis	-1.810	0.839	-5.899	2.279
	Periodontitis	-1.550	1.000	-5.639	2.539
Gingivitis	Periodontitis	0.260	1.000	-3.829	4.349

TABLE 3: Mean Hb values in different groups

Group	Mean	Std Dev	SE of Mean	95% CI for Mean		Min	Max
				Lower Bound	Upper Bound		
Healthy	13.77	1.573	0.352	13.034	14.506	10.9	16.4
Gingivitis	12.93	1.510	0.338	12.223	13.637	10.9	14.8
Periodontitis	12.565	1.306	0.292	11.954	13.176	10.6	14.1

TABLE 4: Comparison of mean Hb values between the groups:

Group I	Group J	Mean Difference (I-J)	P-Value	95% CI	
				Lower Bound	Upper Bound
Healthy	Gingivitis	0.840	0.227	-0.305	1.985
	Periodontitis	1.205	0.036*	0.060	2.350
Gingivitis	Periodontitis	0.365	1.000	-0.780	1.510

\* Denotes significant difference

TABLE 5: Mean ESR values in different groups

Group	Mean	Std. Dev	SE of Mean	95% CI for Mean		Min	Max
				Lower Bound	Upper Bound		
Healthy	16.825	3.667	0.820	15.109	18.541	12.5	21.9
Gingivitis	18.34	4.170	0.932	16.389	20.291	13.8	23.6
Periodontitis	23.04	3.004	0.672	21.634	24.446	18.5	30.0

TABLE 6: Comparison of mean ESR values between the groups

Group I	Group J	Mean Difference (I-J)	P-Value	95% CI	
				Lower Bound	Upper Bound
Healthy	Gingivitis	-1.515	0.582	-4.358	1.328
	Periodontitis	-6.215	<0.001*	-9.058	-3.372
Gingivitis	Periodontitis	-4.700	<0.001*	-7.543	-1.857

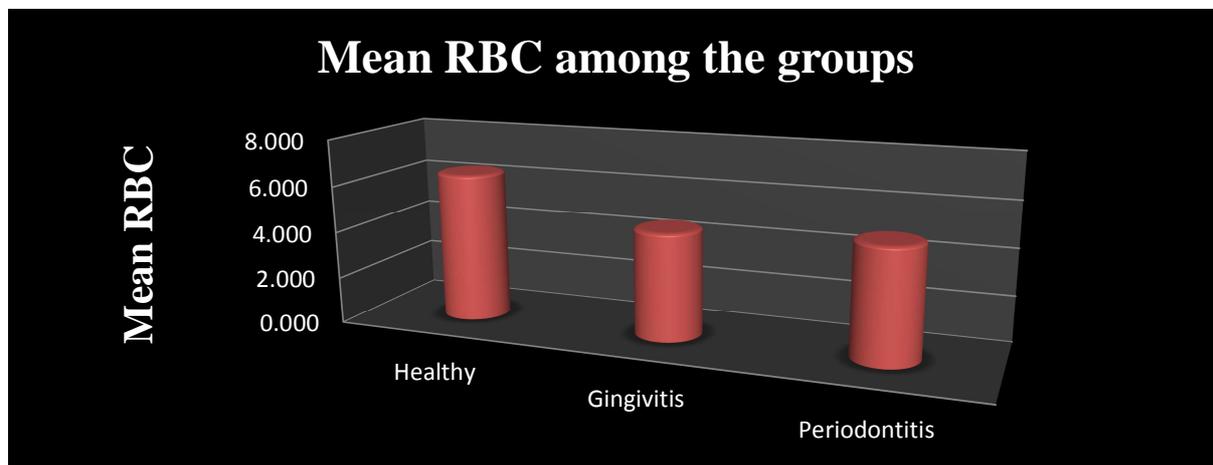
\* Denotes significant difference

**Comparison of RBC between the groups:**

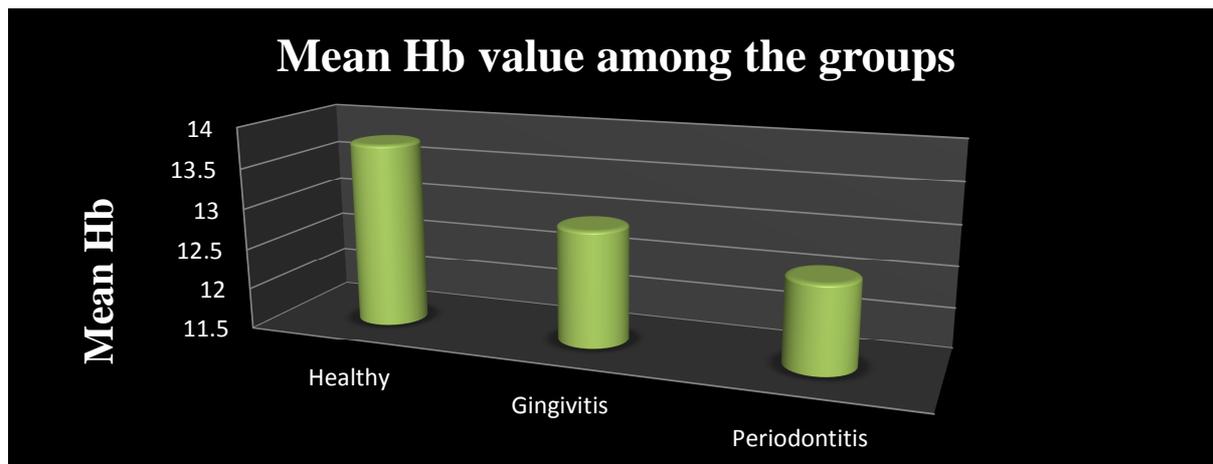
Intergroup comparison of RBC scores have been shown in Table 2. The mean difference in the RBC score between healthy and gingivitis groups were -1.810 with P value of 0.839 ( $P > 0.05$ ). The mean difference in the RBC score

between healthy and periodontitis groups were -1.550 with P value of 1.000 ( $P > 0.05$ ). The mean difference in the RBC scores between gingivitis and periodontitis groups were 0.260 with P value of 1.000 ( $P > 0.05$ ). However, the difference in mean RBC was not statistically significant between any groups ( $P > 0.05$ ).

GRAPH 1



GRAPH 2



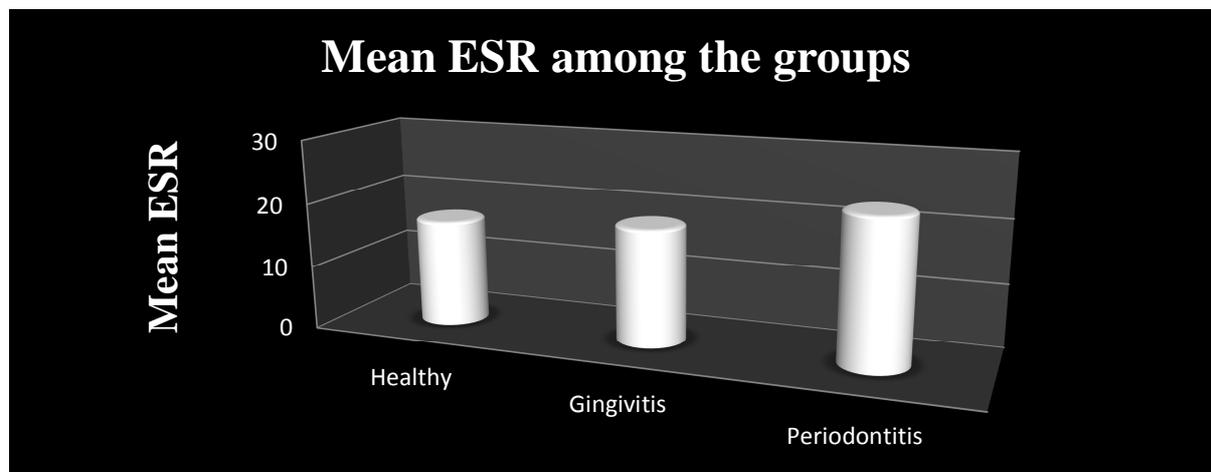
#### Mean Hb among the groups

The mean Hb among all the three groups has been shown in Table 3 and Graph 2. In the healthy group the mean Hb scores were  $13.77 \pm 1.573$ . In the gingivitis group, the mean Hb scores were  $12.93 \pm 1.510$ . In the periodontitis group the mean Hb scores were  $12.565 \pm 1.306$ . The mean Hb values were found to be highest in healthy group, followed by gingivitis group and periodontitis group respectively. The difference in mean Hb values among the three groups were found to be statistically significant ( $P < 0.05$ ).

#### Comparison of Hb between the groups

Intergroup comparisons of Hb scores have been shown in Table 4. The mean difference in the Hb score between healthy and gingivitis groups were 0.840 with P value of 0.227 ( $P > 0.05$ ). The mean difference in the Hb score between healthy and periodontitis groups were 1.205 with P value of 0.036 ( $P < 0.05$ ). The mean difference in the Hb score between gingivitis and periodontitis groups were 0.365 with P value of 1.000 ( $P > 0.05$ ). However the difference in mean Hb values were found to be statistically significant only between healthy and periodontitis groups ( $P < 0.05$ ).

GRAPH 3



#### Mean ESR among the groups

The mean ESR among all the three groups has been shown in Table 5 and Graph 3. In the healthy group the mean ESR scores were  $16.825 \pm 3.667$ . In the gingivitis group the mean ESR scores were  $18.34 \pm 4.170$ . In the periodontitis group the mean ESR scores were  $23.04 \pm 3.004$ . The mean ESR was found to be highest in periodontitis group, followed by gingivitis group and healthy group respectively. The difference in mean ESR among the three groups were found to be statistically significant ( $P < 0.001$ ).

#### Comparison of ESR between the groups

Intergroup comparisons of ESR scores have been shown in Table 6. The mean difference in the ESR score between healthy and gingivitis groups were  $-1.515$  with P value of  $0.582$  ( $P > 0.001$ ). The mean difference in the ESR score between healthy and periodontitis groups were  $-6.215$  with P value of  $< 0.001$ . The mean difference in the ESR score between gingivitis and periodontitis groups were  $-4.700$  with P value of  $< 0.001$ . The difference in mean ESR was found to be statistically significant between healthy and periodontitis groups ( $P < 0.001$ ) as well as between gingivitis and periodontitis groups ( $P < 0.001$ ).

Periodontitis is one of the most common oral and dental health problems currently, and it not only requires clinical attention but also merits further clinical research. As periodontal disorders are known to be associated with several systemic disorders such as diabetes, stroke, neoplasms etc. most patients with periodontitis require further medical evaluation and management of their systemic condition to improve their oral health and to prevent further dental loss. [16] The association of anemia and periodontitis has been explored since the early 20th century. Earlier reports have suggested anemia to be a cause, and not a consequence, of destructive periodontitis. Lainson *et al* [7] was one of the first authors to implicate anemia as a systemic cause of periodontitis. Chawla *et al*. [8] suggested that anemia is an important factor in the etiology or pathogenesis of periodontal disease. Siegel [6] reported a depression in the number of erythrocytes apparently secondary to the presence of periodontal disease. Hutteret *al*. [9] evaluated the blood parameters in patients with chronic periodontitis and concluded that these patients show signs of anemia.

In the present cross sectional study, the hematological parameters like the RBC count, Hb count and the ESR levels were estimated for healthy, gingivitis and periodontitis subjects and were compared in order to determine if any correlation exists between anemia and development of periodontitis. The current study excluded smokers, [17] patients on antibiotics and with history of bleeding disorders, systemic diseases or acute or chronic medical conditions including diabetes, viral, fungal or bacterial infections as these characteristics can act as possible confounding factors that could alter the hematological parameters. [18]

The hematological parameters like hemoglobin (Hb), erythrocyte sedimentation rate (ESR), and RBC were selected for evaluation, as these are indicative of the anemic state of the patient.

Results of the study showed that

1) Mean RBC count was highest in healthy group, followed by periodontitis group and gingivitis group respectively even though the difference in mean RBC among the three groups were not statistically significant ( $P>0.05$ ). The reason for the diminished RBC count in gingivitis and periodontitis subjects may be related to elevated levels of pro-inflammatory cytokines in plasma of these patients suppressing erythropoiesis. [19] When intergroup comparisons were done, no statistically significant differences were seen between any of the groups. The results of the present study were in agreement with those done by Ghokaleet al [13] where the patients with chronic periodontitis had lower values of hematocrit, number of erythrocytes and hemoglobin compared to healthy controls. But contrasting results were given by Shobha Prakash et al [15] in their study where they evaluated levels of hemoglobin, hematocrit, erythrocyte sedimentation ratio, serum iron and ferritin between subjects with and without periodontitis. Except for ESR which was significantly higher in mild periodontitis group than the control group, hematological and biochemical parameters were not significantly different among the study group or between the study and control groups.

2) Mean Hb values were found to be highest in healthy group, followed by gingivitis group and periodontitis group respectively. However the differences in mean values of Hb were found to be statistically significant between healthy and periodontitis groups ( $P<0.05$ ).

The down regulation of the erythropoiesis in bone marrow by pro-inflammatory cytokines such as interleukin (IL 1 and IL 6) and tumor necrosis factor-alpha could be responsible for a decreased number of erythrocytes which in turn reduces the hemoglobin levels in the blood.

3) Mean ESR was found to be highest in periodontitis group, followed by gingivitis group and healthy group respectively. The difference in mean ESR among the three groups was found to be statistically significant ( $P<0.001$ ). When intergroup comparisons were carried out, the difference in mean ESR was found to be statistically significant between healthy and periodontitis groups ( $P<0.001$ ) as well as between gingivitis and periodontitis groups ( $P<0.001$ ).

ESR is a measure of the rate at which erythrocytes sediment in anti-coagulated whole blood under a given set of conditions. Accelerated erythrocyte aggregation is caused by large, asymmetrical plasma proteins (fibrinogen, immunoglobulins, lipoproteins and  $\alpha$ -2 macroglobulin) and inflammation related proteins (cytokines and chemokines) inhibiting the negative electrical forces that normally keep the erythrocytes apart. The reason for elevated ESR in the present study may be due to the chronic inflammatory process associated with periodontitis. The results of the present study was in accordance with a study by Thomas et al [20] who also reported lower hematocrit, lower number of erythrocytes, lower hemoglobin levels and higher erythrocyte sedimentation rates in periodontitis patients. Agarwal et al [21] demonstrated a significant improvement in hemoglobin value and erythrocyte count after periodontal treatment, including surgery in patient with generalized chronic periodontitis with anemia. However, Wakaiet al[22] and Aljohaniet al [23] failed to show any association between hemoglobin levels and periodontal status. A major limitation of the present study is the small sample size used in the study. A study to evaluate the effect of periodontal interventions on hematological parameters has to be carried out to further validate the association between anemia and periodontitis.

Within the limitations of the present study, it is seen that chronic periodontal disease is associated with alterations in the hematological parameters. These findings may be related to the elevated levels of pro-inflammatory cytokines in plasma of periodontitis patients suppressing erythropoiesis. Further studies with a larger study population for a longer period of time are required to confirm the association.

### CONCLUSION

The present cross sectional study was designed to assess the relationship between periodontitis and anemic state by comparing the hematological parameters like RBC, Hb and ESR between periodontally healthy, gingivitis and periodontitis subjects. The results of the study revealed that

- Red Blood Cell count and Hemoglobin levels were reduced in gingivitis and periodontitis subjects compared to the healthy individuals. The reason may be the elevated levels of pro-inflammatory cytokines in plasma of these patients suppressing erythropoiesis.
- Erythrocyte Sedimentation Rates were higher in the periodontitis subjects suggesting an inflammatory process associated with periodontitis.

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