Original Article

Qualitative assessment of red blood cell parameters for signs of anemia in patients with chronic periodontitis

Nubesh S. Khan, Roji Luke[1], Rino Roopak Soman[2], Praveen M. Krishna[3], Iqbal P. Safar[4], Senthil Kumar Swaminathan[5]

Department of Periodontics, Sri Sankara Dental College, Trivandrum, [1]Department of Periodontics, Educare Institute of Dental Sciences, Malappuram, [2]Department of Periodontics, Pushpagiri College of Dental Sciences, Thiruvalla, [3]Department of Periodontics, SRI Ramakrishna Dental College and Hospital, Coimbatore, [4]Department of Periodontics, Malabar Dental College, Edappal, Kerala, [5]Department of Periodontics, Rajah Muthiah Dental College and Hospital, Chidambaram, Tamil Nadu, India

Corresponding author (email: <drnubesh@gmail.com>)
Dr. Nubesh S. Khan, Department of Periodontics, Sri Sankara Dental College, Varkala, Trivandrum, Kerala, India.

Abstract

Aim: Anemia of chronic disease is defined as anemia occurring in chronic infections and inflammatory conditions that is not caused by marrow deficiencies or other diseases and in the presence of adequate iron stores and vitamins. The present case control study was aimed to assess the red blood cell parameters for signs of anemia in patients with mild, moderate, and severe chronic periodontitis. **Materials and Methods:** A simple random sampling method was used to select 80 healthy male patients, who were divided into four groups based on full mouth periodontal examination as follows: group I patients comprised the control group (n = 20), which included patients with a clinically healthy periodontium, group II patients (n = 20) were diagnosed with mild chronic periodontitis, group III (n = 20) included moderate chronic periodontitis patients, and patients with severe chronic periodontitis formed group IV (n = 20). Laboratory blood investigations included total number of erythrocytes, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. **Results:** Data analysis showed a statistically significant decrease in red blood cell parameters with increase in different grades of periodontitis. **Conclusion:** Results of the present study show a substantial decrease in red blood cell parameters with increase in the severity of periodontal destruction.

Key words: *Anemia, chronic periodontitis, cytokines, hemoglobin, red, red blood cells*

INTRODUCTION

Periodontitis is a chronic infectious condition of the supporting tissues of the teeth that is caused by a complex variety of anaerobic, gram-negative bacteria. Periodontal destruction probably results from the action of various toxic products released from specific pathogenic subgingival plaque bacteria, as well as from the host responses elicited against plaque bacteria and their products. The inflammatory response may result in gingival ulceration around the tooth which can allow the entry of intact bacterial cells or their products including lipopolysaccharide, peptidoglycan fragments,

Access this article online	
Quick Response Code:	Website: www.jispcd.org
	DOI: 10.4103/2231-0762.167726

How to cite this article: Khan NS, Luke R, Soman RR, Krishna PM, Safar IP, Swaminathan SK. Qualitative assessment of red blood cell parameters for signs of anemia in patients with chronic periodontitis. J Int Soc Prevent Communit Dent 2015;5:476-81..

and hydrolytic enzymes into the systemic circulation.[1] Although plaque is essential for the initiation of periodontal diseases, the majority of the destructive processes associated with these diseases are due to an extensive host response to the bacterial challenge. Therefore, periodontal disease is a multifactorial, complex disease. The two types of periodontitis are chronic periodontitis and aggressive periodontitis. Characteristic clinical findings in patients with untreated chronic periodontitis include supragingival and subgingival plaque and calculus accumulation, gingival inflammation, pocket formation, loss of periodontal attachment, alveolar bone loss, and occasional suppuration. Aggressive periodontitis differs from the chronic form primarily by the rapid rate of disease progression seen in otherwise healthy individual, absence of large accumulation of plaque and calculus, and a family history of aggressive disease suggestive of a genetic trait. Based on the severity of destruction of the periodontium that occurs as a result of chronic periodontitis, the disease severity can be described as mild, moderate, and severe. In mild periodontitis, periodontal destruction is generally considered slight when there is no more than 1–2 mm of clinical attachment loss. When 3–4 mm of clinical attachment loss has occurred, it is considered as moderate periodontitis. Periodontal destruction is considered severe when 5 mm or more of clinical attachment loss has occurred.

The host response to infection is an important factor in determining the extent and severity of periodontal disease. Epidemiologic studies suggest that periodontal problems increase the risk of systemic problems like cardiovascular diseases,[2] cerebrovascular diseases,[2] atherosclerosis,[3] preterm low birth weight,[4] and diabetes mellitus.[5] These reported associations indicated that periodontal diseases have systemic effects.

The blood cells have a vital role in supplying oxygen, maintaining hemostasis, and providing protection to the tissues of the periodontium. Systemic hematological disorders can thus have profound effects on the periodontium; if any of these functions is altered, it can have a detrimental effect on the integrity of the periodontium.[6] For thousands of years, blood has been regarded as the ultimate body fluid that could indicate disease processes. In the past decade, there has been a renewed interest in the ways in which periodontitis may affect changes in cellular and molecular components of peripheral blood.[7]

Anemia is defined as a state of reduced hemoglobin concentration, reduced number of circulating erythrocytes in the blood, or both.[8] Anemia of chronic disease (ACD) is the most common form of anemia observed in clinical medicine.[9,10] The ACD is defined as the anemia that occurs in chronic infections, inflammatory conditions, or neoplastic disorders that is not due to marrow deficiencies or other diseases and occurs despite the presence of adequate iron stores and vitamins.[11,12]

The purpose of the present study was to compare the hematological parameters related to anemia in male patients with mild, moderate, and severe chronic periodontal disease with those of periodontally healthy male subjects and, thus, evaluate a possible association between periodontal disease and anemia.

MATERIALS AND METHODS

Study population

This study was conducted in March 2011 in the outpatient Department of Periodontology and Implantology, Rajah Muthiah Dental College and Hospital, Tamil Nadu. India. A simple random sampling method was used to select 80 healthy male patients, aged–55 years, in the study. Group I was the control group consisting of 20 male subjects with clinically healthy gingival. The test group included 60 male patients who were divided into three groups with 20 patients in each. Twenty male patients with probing pocket depth of greater than 4 mm and clinical attachment level (CAL) of 1–2 mm were diagnosed with mild chronic periodontitis. These patients made up group II. Group III included 20 male patients with probing pocket depth of greater than 4 mm and CAL of 3–4 mm, and were diagnosed with moderate chronic periodontitis. Twenty male patients with probing pocket depth of greater than 4 mm and CAL of 5 mm, who were diagnosed with severe chronic periodontitis made up group IV. All patients received verbal explanation of the nature of the study, and informed written consent was obtained. The study was approved by the Medical Ethical Committee of Rajah Muthiah Dental College and Hospital.

A detailed systemic and family history was recorded. Patients with a history of systemic diseases or conditions that may adversely affect periodontal health were excluded from the study. The exclusion criteria for the study were as follows: (1) Female patients; (2) patients with blood disorders and recent history of blood transfusion; (3) patients with history of diabetes, kidney disease, cancer, or fungal or respiratory infections; (4) patients with a history of

hospitalization or intake of medications in the last (6 months; (5) patients with a current or past habit of tobacco smoking or chewing; and (6) patients with a previous history of periodontal therapy.

Clinical recordings

Oral hygiene was recorded using simplified oral hygiene index (OHI-S). Gingival inflammation was scored using gingival index (GI), probing depth (PD), and CAL measures obtained from the four points of the teeth using a conventional periodontal probe (Hu-Friedy, Chicago, IL, USA) [Figure 1]. The probe was directed parallel to the long axis of the tooth. CAL measurements were made from the cemento-enamel junction to the bottom of the sulcus [Figure 2]. All clinical data were recorded by one examiner.

Blood collection and analysis

Two milliliters of venous blood samples was collected by venipuncture in the antecubital fossa under aseptic conditions without excessive venous stasis from both case and control group patients. The blood was transferred into ethylenediaminetetraacetic acid (EDTA) containing bulbs and processed within 4 h of collection in an automated hematological analyzer. The hematological parameters assessed in the present study were the number of erythrocytes, mean corpuscular volume (MCV), hemoglobin concentration, hematocrit (packed cell volume), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Statistical analysis

All the data were analyzed using SYSTAT, 12 software program. Means ± SD of all the parameters was calculated for all the four groups. Analysis of variance (ANOVA) statistical test was applied to compare the mean values of red blood cell parameters in all the four groups. Pearson correlation coefficient test was applied to correlate the red blood cell parameters with clinical parameters.

Figure 1: Conventional periodontal probe

RESULTS

On average, 28 teeth were present in all the four groups. The mean ± SD of all blood parameters in the test and control groups are presented in Table 1.

The mean number of erythrocytes and the hemoglobin values were significantly lower in mild (5.1 ± 0.2 million/mm^3 and 14.5 ± 0.3 g%, respectively), moderate (4.8 ± 0.2 million/mm^3 and 13.5 ± 0.2 g%, respectively), and severe (4.4 ± 0.3 million/mm^3 and 12 ± 1.0 g%, respectively) periodontitis groups, when compared to the healthy controls (5.2 ± 0.3 million/mm^3 and 15.2 ± 0.3 g%, respectively). The difference was found to be highly significant (P = 0.0001). Similarly, the mean values for PCV and MCV were significantly lower in group II (43.5 ± 6.8% and 91 ± 3.1 fl, respectively), group III (42.7 ± 1.5% and 88.0 ± 4.9 fl, respectively), and group IV (38.0 ± 4.0% and 85.1 ± 9.2 fl, respectively), when compared to group I (48.2 ± 2.2% and 91.1 ± 3.1 fl, respectively), and the difference was statistically significant (P < 0.001). The mean values of MCH and MCHC were noted to be higher in group I (30.1 ± 1.5 pg and 32.7 ± 1.3 g/dl, respectively) compared to group II (29.0 ± 1.1 pg and 32.2 ± 0.8 g/dl, respectively), group III (27.6 ± 1.7 pg and 31.5 ± 0.8 g/dl, respectively), and group IV (26.6 ± 3.3 pg and 31.8 ± 1.2 g/dl, respectively). The difference was found to be highly significant (P = 0.0001) [Table 1].

Pearson correlations between red blood cell parameters and clinical parameters are shown in Table 2. A significant negative correlation was observed between RBC, hemoglobin, PCV, MCV, MCH, and MCHC with the clinical parameters studied.

Figure 2: CAL measurements from the cemento-enamel junction to the bottom of the sulcus

Table 1: Mean, SD, and Student's *t*-test significance values for systemic parameters within the four groups

Parameters	Mean±SD				*P**
	Group I	Group II	Group III	Group IV	
Number of erythrocytes (million/mm^3)	5.2±0.3	5.1±0.2	4.8±0.2	4.4±0.3	<0.001
Hemoglobin (g%)	15.2±0.3	14.5±0.3	13.5±0.2	12±1.0	<0.001
PCV (%)	48.2±2.2	43.5±6.8	42.7±1.5	38.0±4.0	<0.001
MCV (fl)	91.1±3.1	90.2±3.7	88.0±4.9	85.1±9.2	<0.001
MCH (pg)	30.1±1.5	29.0±1.1	27.6±1.7	26.6±3.3	<0.001
MCHC (g/dl)	32.7±1.3	32.2±0.8	31.5±0.8	31.8±1.2	<0.001

**P<0.001 denotes statistical significance, SD=Standard deviation. MCV=Mean corpuscular volume, MCH=Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobin concentration, PCV=Packed cell volume*

Table 2: Pearson correlation coefficient (test to compare RBC, hemoglobin, PCV, MCV, MCH, and MCHC with clinical parameters)

	RBC	HB	PCV	MCV	MCH	MCHC
OHI-S	−0.590	−0.773	−0.546	−0.316	−0.436	−0.422
Gingival index	−0.617	−0.803	−0.597	−0.333	−0.450	−0.407
Russel's index	−0.603	−0.837	−0.609	−0.382	−0.499	−0.415
Mean PPD	−0.636	−0.844	−0.575	−0.382	−0.484	−0.394
CAL	−0.660	−0.877	−0.619	−0.393	−0.508	−0.423

MCV=Mean corpuscular volume, MCH=Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobin concentration, CAL=Clinical attachment level, OHI-S=Simplified oral hygiene index, PCV=Packed cell volume, RBC=Red blood cell, PPD=Probing pocket depth

DISCUSSION

Anemia is one of the most common global public health problems in developed and developing countries. Globally, anemia affects 1.62 billion people, which is 24.8% of the total population.[13] ACD is the second most prevalent form of anemia after nutritional, iron-deficiency anemia, and can coexist together, causing additional anemic burden. ACD is a cytokine-mediated anemia characterized by hypoferremia, with adequate reticuloendothelial iron stores and normal-to-elevated ferritin concentrations. It is a known, frequent complication of chronic inflammatory conditions[14,15] such as rheumatoid arthritis. The pathogenesis is reported to be dysregulation of iron homeostasis, depressed erythropoiesis, and a blunted erythropoietin response caused by elevated levels of systemically circulating pro-inflammatory cytokines that are the result of a local chronic inflammatory process.

The relation between anemia and periodontitis was explored in the latter half of the 20th century. The studies investigated both the concept of anemia as an etiological factor of periodontitis and periodontitis as a risk factor for anemia. It was hinted and believed that anemia may be a factor in the causation of periodontitis, rather than a consequence.[16] Lainson *et al*.[17] was one of the first authors to implicate anemia as a systemic cause of periodontitis. Chawla *et al*.[18] suggested that anemia is an important factor in the etiology or pathogenesis of periodontal disease. In anemic patients, there is increased production of the cytokines that mediate the immune or inflammatory response; such as tumor necrosis factor, interleukin-1, and the interferon. All the processes involved in the development of anemia can be attributed to these cytokines, including shortened red cell survival, blunted erythropoietin, and abnormal mobilization of reticuloendothelial iron stores. These cytokines are also released by periodontal tissues in response to bacterial infection, which suggests that periodontitis, like other chronic diseases, may cause anemia.

Siegel[19] reported a depression in the number of erythrocytes apparently secondary to the presence of periodontal disease.

Recently, various studies have tried to evaluate the relation between periodontitis and anemia. These studies present confounding results. Hutter *et al*.[20] and Thomas *et al*.[21] showed that periodontitis patients have a lower hematocrit, lower number of erythrocytes, lower hemoglobin levels, and higher erythrocyte sedimentation rates. A 10-week intervention study by Rai and Kharb[22] found an increase in hemoglobin and RBC levels after scaling and root planing in patients with severe periodontitis. Agarwal *et al*.[23] demonstrated a significant improvement in hemoglobin value and erythrocyte count after periodontal treatment, including surgery in patients with generalized chronic periodontitis with anemia. A study by Gokhale *et al*.[24] showed that patients suffering from chronic periodontitis have a lower number of erythrocytes and hemoglobin compared to healthy controls. Thus, they concluded that like any other chronic condition, chronic periodontitis can lead to anemia.

In the present study, tobacco smokers and chewers were excluded because they present confounding factors.

Smoking affects the immune system and microflora of the patients, leading to deeper PDs[25] and greater clinical attachment[26] and bone loss.[27] Smoking has a greater effect on the release of cytokines from neutrophils than periodontal disease.[28] Smoking also affects the erythrocytes and other blood parameters.[29] According to a study by Erdemir *et al*.,[30] smokers with chronic periodontitis have a lower number of erythrocytes, a lower value of hemoglobin, and lower hematocrit and iron, compared to non-smokers with chronic periodontitis.

In India, anemia is more prevalent in females because of poor nutrition, increased menstrual losses, high incidence of tropical and intestinal infections, and other miscellaneous factors. Iron deficiency anemia is the most common type of anemia seen in India.[31] Females are also prone to hormonal imbalance during puberty, during the reproductive phase, and toward menopausal age. The microbial flora and host immune response are altered leading to exaggerated response of the periodontal tissues to local factors.[32] Therefore, to eliminate bias, only male patients were included in the study. Since our present study assesses the hematological parameters, patients having blood disorders like anemia, leukemia, lymphoma, and bleeding disorders like hemophilia, von Willebrand disease, etc., were excluded.

The present study shows an association between chronic periodontitis and signs of anemia, which suggests a measurable effect of periodontitis on the systemic condition of the patient. The mean hematocrit value was significantly lower in patients with mild, moderate, and severe periodontitis compared to healthy controls. The lower value of hematocrit can be attributed to the significantly lower number of erythrocytes. Similarly, the mean hemoglobin value was significantly lower in the test group. Our findings are similar to those reported previously.[20] In our study, the mean MCV, MCH, and MCHC values were found to be higher in healthy controls when compared to the test group. The current observations were in accordance with the findings of Naik *et al*.[33]

Cartwright[34] postulated that at least three pathologic processes are involved in ACD: (1) Shortened erythrocyte survival, (2) failure of the bone marrow to increase RBC production to compensate for this increased demand, and (3) impaired release of iron from the reticuloendothelial system. The proinflammatory cytokines are thought to act as mediators in suppressing erythropoiesis from the bone marrow leading to anemia. The severity of anemia may depend on the elevation of proinflammatory cytokines. The change in hemoglobin and RBC values in the present study was statistically significant, but the difference was not as striking as observed in anemia caused by other inflammatory conditions, such as rheumatoid arthritis, neoplastic conditions, and fungal or parasitic infections. This may be explained by the fact that chronic periodontitis is a milder inflammatory condition compared to other systemic infections or conditions. Further studies need to be conducted to correlate the amount of periodontal inflammation and its effects on the systemic health of an individual.

CONCLUSION

Systemic infections, especially chronic conditions, have a direct effect on the general health and well-being of an individual. Chronic diseases, such as rheumatoid arthritis, have been associated with anemia. Chronic periodontitis leads to a low-grade systemic inflammation. The long-standing inflammatory change in the periodontium has been associated with altered diabetic control, preterm low birth weight, and cardiovascular disease. Thus, chronic periodontitis has definite systemic effects. Chronic periodontitis can also lead to signs of anemia. Being a low-grade infection, the signs may not be as severe as seen in other systemic conditions, but they definitely cannot be ignored.

Acknowledgments

The authors wish to acknowledge the Department of Periodontics, Rajah Muthiah Dental College and Hospital, Chidambaram, Tamil Nadu, India for providing support for conducting the study. This study was self-funded by the authors and their institutions. The authors report no conflicts of interest related to this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Prathap S, Hegde S, Rajesh KS, Arun MS. Anemia-risk factor for periodontitis. KDJ 2010;33:173-5.
2. Mustapha IZ, Debrey S, Oladubu M, Ugarte R. Markers of systemic bacterial exposure in periodontal disease and cardiovascular disease risk: A systematic review and meta-analysis. J Periodontol 2007;78:2289-302.

3. Sim SJ, Kim HD, Moon JY, Zavras AI, Zdanowicz J, Jang SJ, *et al*. Periodontitis and the risk for non-fatal stroke in Korean adults. J Periodontol 2008;79:1652-8.
4. Wimmer G, Pihlstrom BL. A critical assessment of adverse pregnancy outcome and periodontal disease. J Clin Periodontol 2008;35(Suppl):380-97.
5. Mealey BL, Rose LF. Diabetes mellitus and inflammatory periodontal diseases. Compend Contin Educ Dent 2008;29:402-8, 410, 412-3.
6. Kinane DF, Marshall GJ. Periodontal manifestations of systemic disease. Aust Dent J 2001;46:2-12.
7. Loos BG. Systemic markers of inflammation in periodontitis. J Periodontal 2005;76(Suppl):2106-15.
8. Enhos S, Duran I, Erdem S, Buyukbas S. Relationship between iron-deficiency anemia and periodontal status in female patients. J Periodontol 2009;80:1750-5.
9. Lee GR. The anemia of chronic disease. Semin Hematol 1983;20:61-80.
10. Beutler E. The common anemias. JAMA 1988;259:2433-7.
11. Means RT Jr, Krantz SB. Progress in understanding the pathogenesis of the anemia of chronic disease. Blood 1992;80:1639-47.
12. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med 2005;352:1011-23.
13. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr 2009;12:444-54.
14. Means RT. Hepcidin and cytokines in anaemia. Hematology 2004;9:357-62.
15. Lea JP, Norris K, Agodoa L. The role of anemia management in improving outcomes for African-Americans with chronic kidney disease. Am J Nephrol 2008;28:732-43.
16. Agarwal N, Kumar VS, Gujjari SA. Effect of periodontal therapy in hemoglobin and erythrocyte levels in chronic generalized periodontitis patients: An interventional study. J Indian Soc Periodontol 2009;13:6-11.
17. Lainson PA, Brady PP, Fraleigh CM. Anemia, a systemic cause of periodontal disease? J Periodontol 1968;39:35-8.
18. Chawla TN, Kapoor KK, Teotia SP, Singh NK. Anaemia and periodontal disease - a correlative study. J Indian Dent Assoc 1971;43:67-78.
19. Siegel EH. Total erythrocyte, leukocyte and differential white cell counts of blood in chronic periodontal disease. J Dent Res 1945;24:270.
20. Hutter JW, van der Velden U, Varoufaki A, Huffels RA, Hoek FJ, Loos BJ. Lower numbers of erythrocytes and lower levels of hemoglobin in periodontitis patients compared to control subjects. J Clin Periodontol 2001;28:930-6.
21. Thomas B, Ramesh A, Rithesh K. Relationship between periodontitis and erythrocyte count. JISP 2006;10:288-91.
22. Rai B, Kharb S. Effect of scaling and root planing in periodontitis on peripheral blood. Int J Dent Sci 2008;6.
23. Agarwal N, Kumar VS, Gujjari SA. Effect of periodontal therapy on hemoglobin and erythrocyte levels in chronic generalized periodontitis patients: An interventional study. J Indian Soc Periodontol 2009;13:6-11.
24. Gokhale SR, Sumanth S, Padhye AM. Evaluation of blood parameters in patients with chronic periodontitis for signs of anemia. J Periodontol 2010;81:1202-6.
25. Bergström J. Tobacco smoking and risk for periodontal disease. J Clin Periodontol 2003;30:107-13.
26. Axelsson P, Paulander J, Lindhe J. Relationship between smoking and dental status in 35-, 50-, 65-, and 75-year-old individuals. J Clin Periodontol 1998;25:297-305.
27. Bergström J, Eliasson S, Dock J. A 10-year prospective study of tobacco smoking and periodontal health. J Periodontol 2000;71:1338-47.
28. Tappia PS, Troughton KL, Langley-Evans SC, Grimble RF. Cigarette smoking influences cytokine production and antioxidant defences. Clin Sci (Lond) 1995;88:485-9.
29. Ernst E. Haemorheological consequences of chronic cigarette smoking. J Cardiovasc Risk 1995;2:435-9.
30. Erdemir EO, Nalcaci R, Caglayan O. Evaluation of systemic markers related to anemia of chronic disease in the peripheral blood of smokers and non-smokers with chronic periodontitis. Eur J Dent 2008;2:102-9.
31. Medappa N. Iron absorption and its implications on strategies to control iron deficiency anaemia. Indian Councs Med Res Bull 2000;30:1-7.
32. Amar S, Chung KM. Influence of hormonal variation on the periodontium in women. Periodontol 2000 1994;6:79-87.
33. Naik V, Acharys A, Deshmukh VL, Shetty S, Shirhatti R. Generalized, severe, chronic periodontitis is associated with anemia of chronic disease: A pilot study in urban, Indian males, J Investig Clin Dent 2010;1:139-43.
34. Cartwright GE. The anemia of chronic disorders. Semin Hematol 1966;3:351-75.