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Original Research Article

Comparison of red blood cell parameters in patients with and without periodontitis – A hospital based observational study

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ABSTRACT

Background: Dental plaque bio film aggregate around dentition is the primary etiology of periodontitis. The host inflammation mounted in response to bacterial biofilm determines the extent and severity of periodontitis. The low grade inflammation results increase in pro inflammatory cytokine levels, and this links periodontitis with other systemic diseases. Epidemiologic studies have revealed a possible association of periodontitis with some systemic diseases. There are conflicting results regarding the association of periodontitis and anemia. The aim of the study was to compare the red blood cell parameters in subjects with and without periodontitis in our population.

Materials and Methods : A single calibrated examiner was entrusted to do the periodontal examination, and 48 subjects with periodontitis (Group I) and 50 age matched subjects without periodontitis (Group II) with a mean age of 33.45 yrs. +/- 7.86 were recruited for the study. Gingival index and full mouth periodontal charting were done and 2ml blood samples were collected for estimation of blood parameters.

Results: Group I showed a statistically insignificant difference in hemoglobin levels compared to group II (P value-0.065) where as MCH and MCHC showed a statistically significant difference between the groups (P value-0.012, 0.002 respectively).

Conclusion: The inflammatory burden increases with the severity of Periodontitis and this may lead to the development of anemia.

1. Introduction

Periodontitis, a globally common disease, begins with a dysbiotic oral biofilm which naturally forms on teeth and surrounding gingival area. The magnitude of inflammatory response and the differential activation of immune pathways determine the extent and severity of periodontal destruction.[1] The ulcerated sulcular epithelium acts as a portal of entry of bacteria and their noxious products to the underlying connective tissue and to the systemic circulation. Periodontal inflammation induces a low-grade systemic inflammation as evidenced by increase in acute phase proteins like C reactive protein (CRP).[2] Literature evidence has suggested a possible link between periodontitis and systemic diseases like coronary artery disease and diabetes.[3,4]

Anemia is another common disease of serious concern, prevalent mainly in undeveloped and developing countries. Anemia of Chronic Disease (ACD) also known as Anemia of inflammation (AI), is a form of anemia occurring in

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chronic infection or chronic inflammatory process or tumor formation that is not due to dysfunction of bone marrow cells or other disease, and occurring in spite of adequate iron stores and vitamins.[5] The pro inflammatory cytokines, tumour necrosis factor (TNF), interleukins (IL)-1 and 6, and interferon (IFN) are involved in the development of ACD, though the mechanism is not completely understood.[6,7]

Since periodontitis is a chronic inflammatory disease, with high expression of pro inflammatory cytokines, it is possible that the systemic inflammation and the production of inflammatory mediators induced by periodontal infection may lead to the development of anemia.

Various studies reported in literature have evaluated the blood parameters of periodontitis patients. Majority of these studies have shown statistically significant difference in these parameters in patients with and without periodontitis, but the results are conflicting. A recent systematic review and meta-analysis concluded that periodontitis decreases hemoglobin concentration and disturbs the balance of iron metabolism, which confirms the strength of association between periodontitis and the development tendency of ACD, especially in severe periodontitis.[8] The authors suggested that more unbiased cohort studies with large sample size have to be conducted to make a definite judgement regarding the relationship.

Very few studies on the relationship between periodontitis and ACD have been conducted in our population. Hence the aim of the present study was to evaluate and compare the hematological parameters in patients with and without periodontitis among patients attending a tertiary dental care hospital, Kerala.

2. Materials and Methods

The hospital based comparative cross-sectional study was conducted in the outpatient department of Periodontology in a Government tertiary care center after obtaining clearance from institutional ethics committee. We hypothesized that red blood cell parameters (hemoglobin levels, RBC count, MCV, MCH, MCHC, and ESR) differs in patients with and without periodontitis. The sample size was calculated using the formula $n = (Z_a + Z_b)^2 \times SD^2 \times 2 \div d^2$. Type 1 error was kept as 0.05 and type 2 error as 0.20. Substituting the values from a previous reference study[9] and considering the chance of 10% dropouts during the study, the minimum sample size was calculated as 48 in each group.

Males in the age group between 20-55 years, with at least 20 teeth and who satisfied the inclusion criteria were consecutively age matched and selected for the study. Among them, 48 participants had periodontitis (Group I, Periodontitis group) and 50 participants were without periodontitis (Group II, Control group) according to criteria put forward by the World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions, 2017.[10] All participants were informed about the study protocol and written consent was obtained. Personal and clinical data were collected using proformas. Periodontal examination was carried out by a single caliberated examiner using UNC-15 probe. Gingival index and full mouth periodontal charting (probing depth (PD) and Clinical attachment level (CAL) were recorded. Those participants who had any systemic diseases, with a history of hospitalization in past 6 months, intake of systemic antibiotics in past 3 months, regular use of anti-inflammatory drugs, with a history of periodontal therapy within 6 months, with a history of blood loss, blood transfusion with in last 6 months and chronic tobacco or alcohol use were excluded from the study.

Peripheral blood sample (2 ml) was collected from all participants by venepuncture from the antecubital fossa under complete aseptic conditions. Hemoglobin (Hb), Red blood cell (RBC) count, Mean corpuscular volume (MCV), Mean corpuscular Hemoglobin (MCH), Mean corpuscular Hemoglobin concentration (MCHC) were analyzed on a fully automated hematologic analyzer. The Erythrocyte sedimentation rate (ESR) was measured according to the Wintrobe's method.

Data were analyzed using Kolmogorov-Smirnov test for normality. Data were expressed as mean and standard deviation and analyzed using Computer software SPSS version 20. Unpaired t -test was used to compare hemoglobin levels between the groups. Comparison of RBC count, MCV, MCH, MCHC and ESR between the groups were done by Mann Whitney U test. The association of education with RBC parameters was tested using Analysis of variance (ANOVA). Spearman Correlation was used to assess the correlation between age and RBC parameters. The correlation between gingival inflammation and blood parameters were done using Correlation coefficient. For every comparison, $P < 0.05$ was considered statistically significant.

3. Results

A total of 98 males (48 with periodontitis and 50 without periodontitis) with a mean age of 33.45 yrs. +/- 7.861were age matched and recruited for the study. The descriptive statistics of both the groups is presented in Tables 1 and 2. Since the Hb showed a normal distribution, Unpaired t-test was done to compare Hb levels between the groups. P value was 0.065 and it was not statistically significant (Table 3). Comparison of all other RBC parameters between the groups was done using Mann Whitney U test (Table 4, Graph 1). MCH (amount of hemoglobin present in a single RBC) and MCHC (amount of hemoglobin per unit volume of RBC) showed a statistically significant difference between the groups (P value 0.012 and 0.002 respectively). Other RBC parameters RBC count, mean corpuscular volume and ESR did not show any statistically significant difference between the groups.

Table 1: Descriptive statistics of group 1(Periodontitis group)

Group I	N	Minimum	Maximum	Mean	Std. Deviation
RBC count	48	4.080	5.800	4.97979	.469606
Hb	48	12.000	16.700	14.16250	1.152726
MCV	48	77.400	96.400	81.37083	3.492786
MCH	48	26.000	33.300	27.87500	1.185282
MCHC	48	31.500	35.800	33.55417	.906937
ESR	48	4	50	13.31	10.051
PPD	48	2.250	5.130	3.63708	.583894
CAL	48	2.630	5.650	3.89604	.669130
GI 48	1.000	2.080	1.74167	.305477	
Valid N (listwise)	48				

Table 2: Descriptive statistics of group II (Control group)

Group II	N	Minimum	Maximum	Mean	Std. Deviation
RBC count	50	4.120	5.790	5.01240	.370441
Hb	50	12.800	17.100	14.56800	.985702
MCV	50	77.600	88.700	80.49800	1.781021
MCH	50	25.300	31.300	28.35000	1.109376
MCHC	50	32.100	36.400	34.13600	1.056710
ESR	50	3	28	10.34	6.002
PPD	50	1.240	2.670	2.05780	.284707
CAL	50	.000	.000	.00000	.000000
GI	50	.540	1.580	.95780	.192017
Valid N (listwise)	50				

Table 3: Comparison of hemoglobin (Hb) levels between groups

RBC parameter	Group	N	Mean	Std. Deviation	Std. Error Mean	Mean difference	p value
Hb	Periodontitis group	48	14.16	1.15	0.17	-0.406	0.065
	Control group	50	14.57	0.99	0.14		

Table 4: Comparison of RBC parameters between Periodontitis and control groups

RBC parameters	Group	N	Mean	Std. Deviation	Std. Error Mean	Mean Difference	p value
RBC count	Periodontitis group	48	4.98	0.47	0.07	-0.033	0.672
	control group	50	5.01	0.37	0.05		
MCV	Periodontitis group	48	81.37	3.50	0.50	0.873	0.574
	control group	50	80.50	1.78	0.25		
MCH	Periodontitis group	48	27.88	1.19	0.17	-0.475	0.012*
	control group	50	28.35	1.11	0.16		
MCHC	Periodontitis group	48	33.55	0.91	0.13	-0.582	0.002*
	control group	50	34.14	1.06	0.15		
ESR	Periodontitis group	48	13.31	10.05	1.45	2.97	0.206
	control group	50	10.34	6.00	0.85		

p<0.05* statistically significant

Table 5: Association between RBC parameters and education

(I) Education	(J) Education	Mean Difference (I-J)	Std. Error	Sig.
High school	Graduate	-.622643	.297724	.117
	Pg	-.877679*	.351742	.043
Graduate	High school	.622643	.297724	.117
	Pg	-.255035	.268180	1.000
Pg	High school	.877679*	.351742	.043*
	Graduate	.255035	.268180	1.000

Table 6: Correlation between age and hemoglobin

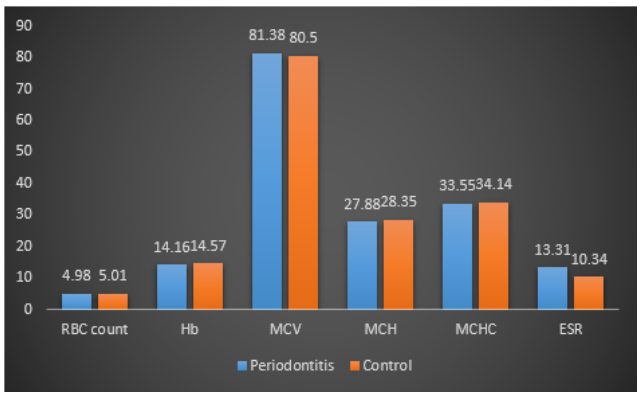
Spearman correlation		Age	Hb
Age	Spearman Correlation	1	-.341
	Sig. (2-tailed)		.018*
		48	48

Table 7: Correlation between age and ESR

Spearman correlation		Age	ESR
Age	Spearman Correlation	1	.349
	Sig. (2-tailed)		.015*
		48	48

Table 8: Correlation betweenblood parameters and gingival inflammation

		RBC count	GI	Hb	MCV	MCH	MCHC	ESR
GI	Correlation Coefficient	-.134	1.000	-.234*	.095	-.203*	-.295**	.199*
	Sig. (2-tailed)	.187	.	.020*	.354	.045*	.003*	.049
		98	98	98	98	98	98	98



Graph 1: Comparison of RBC Parameters among periodontitis and control group

When the association of the parameters (Age, Socio economic status (SES), Education, Diet) were considered with RBC parameters in all 98 subjects, Haemoglobin showed an association (Table 5), which was tested using ANOVA. Hemoglobin showed a positive association with education which was statistically significant (P = 0.043). In both the groups those subjects with higher education, with post-graduation, had higher Hb than those subjects with high school education (Table 5).

When the correlation of age with hemoglobin was assessed, a significant negative correlation was found. For every unit increase in age, Hb level decreased by 0.34 units (P =0.018) (Table 6). Correlation of age with ESR was assessed and a significant positive correlation was found. For every unit increase in age, ESR increased by 0.349 (P = 0.015) (Table 7).

When the correlation between gingival inflammation and RBC parameters was assessed, it was found that a significant negative correlation exists between GI and Hb count, MCH, and MCHC (Table 8). This implies that for every unit increase in gingival index score, there is a decrease of 0.234 units in Hb level, 0.203 units in MCH, and 0.295 units in MCHC levels. The results were found to be statistically significant with a p value less than 0.05.

4. Discussion

The reemergence of focal infection theory and the development of periomedicine as a specialty has thrown light on the facts of increasing association between periodontal disease and systemic conditions. There is ample evidence connecting the bidirectional link between periodontitis and diabetes. Similarly, several studies have confirmed the association between periodontitis and coronary artery diseases. The association between anemia and periodontitis has been an area of interest for researchers for many years. Several studies on the association between anemia and periodontitis have been conducted with conflicting results. Initially it was suggested that anemia is a cause and not a consequence of destructive periodontitis.[11]

The prevalence of mild anemia is 33 to 60 % in Rheumatoid arthritis, a chronic inflammatory disease whose pathogenesis is similar to periodontitis.[12] Many chronic bacterial, fungal and viral infections were reported to have signs of anemia. Anemia, seen in all those chronic conditions are together called “Anemia of chronic diseases” (ACD).[13] Since the chronic conditions and chronic infections precipitate anemia in later life, there is possibility of development of anemia in periodontal disease also. The immuno-inflammatory reactions that develop in response to periodontal infections produces pro inflammatory cytokines TNF-α, IL-1 and IL-6 and lead to decrease in the production of erythropoietin which lead to

the development of anemia.[14]

Siegel was the first person to report a decrease in the number of erythrocytes in periodontitis patients.[15] Hutter et al in their study showed that periodontitis may tend towards the development of anemia as evidenced by lower hematocrit, lower numbers of erythrocytes, lower hemoglobin levels and higher erythrocyte sedimentation rates.[16] Another study by Sneha et al. concluded that chronic periodontitis could lead to signs of anemia and being a low-grade infection, the signs may not be as severe as seen in other systemic conditions, but they definitely cannot be ignored.[17] Vidya Naik, et al. in their study concluded that systemic effects of periodontal inflammation leads to mild anemia of chronic disease in patients with severe periodontal disease.[18] Definite association between periodontal disease and hematological parameters in terms of reduced Hb concentration was noted in the study conducted by Saritha Parihar et al.[9]

There are studies which showed no association between periodontal diseases and anemia. Gayathri et al. concluded that chronic periodontitis being a milder inflammatory condition may not be enough to show drastic changes in hematological status in periodontitis patients.[19] Aljohani H et al in their study also found no association between periodontal diseases and hemoglobin level.[20] Enhos S et al in their study in female patients found no association between anemia and periodontitis.[21]

In the present study the mean hemoglobin in periodontitis group was 14.16 +/_ 1.15 and 14.57 +/_ 0.99 in control group. Though the hemoglobin level difference between the groups was not statistically significant and both groups had hemoglobin levels in the normal range, it should be noted that the mean hemoglobin levels in periodontitis group was comparatively low. Hemoglobin per RBC, MCH and MCHC showed a statistically significant difference between the groups. Other parameters RBC count, MCV, ESR all showed values with in normal range and did not show a statistically significant difference between the groups. The current study included periodontitis patients with a mean probing depth of 3.64 and a mean CAL of 3.89 (stage 1 or stage II periodontitis). So, the results obtained in the present study could be due to the initial stage of the disease which may not result in the decrease of hemoglobin levels and frank development of anemia. But since the MCH and MCHC showed a statistically significant reduction between the groups it is possible that as the severity of periodontitis increases with more number of deep pockets and gingival inflammation, it may lead to signs of anemia as measured by decrease in the hematological parameters.

Studies have evaluated the effects of nonsurgical periodontal therapy in the improvement of haematological parameters. M.D. Patel et al. in their study concluded that like any other chronic condition, chronic periodontitis can lead to ACD and provides evidence that non-surgical periodontal therapy can improve the anemic status of patients with chronic periodontitis.[22] Siddheshapa concluded that there is a decrease in the hematological parameters after nonsurgical periodontal therapy, which may also reduce the risk of atherosclerosis formation in the blood vessel and possibly prevent cardiovascular diseases.[23]

ESR showed a positive correlation with age in this study, for every unit increase in age ESR increased by 0.349. In a study conducted in Rheumatiod arthritis patients it was found that for each decade increase in age, the ESR changes by 1.19.[24] A study conducted in 2019 in Spanish population concluded that ESR increases with age.[25] The results seen in our study is in accordance with the results of previous studies. The acute phase reactants tend to increase with age, and this can probably cause an increase in ESR with age.[24]

Another important finding in our study was a significantly negative correlation of GI with the levels of Hb, MCH, and MCHC. For every unit increase in gingival index score, there was a decrease of 0.234 units in Hb level, 0.203 units in MCH, and 0.295 units in MCHC levels. Chronic low grade inflammation as in case of periodontitis, may results in decreased iron uptake despite of adequate iron stores, decreased response of erythropoietin to bone marrow and or shortened life span of RBCs.[26] A previous study by Anumolu et al. reported a decrease in hemoglobin and erythrocyte count in patients with chronic generalized periodontitis compared to chronic gingivitis patients and healthy controls.[27]

More studies in individuals having gingivitis and different stages of periodontitis with adequate sample size, more specific markers for detection of anemia are recommended in future to make a definite association between anemia and periodontitis.

5. Conclusion

Periodontitis is a chronic bacterial infection which has a definite systemic influence. The possibility of development of anemia as the severity of gingival and periodontal inflammation increases cannot be neglected. The present study could find significant difference in Hemoglobin per RBC (MCH and MCHC) in the periodontitis group. Similarly gingival inflammation showed a negative correlation with Hb count, MCH and MCHC. Patients with severe gingival inflammation should be warned about the systemic effects and the importance of regular dental and medical checkup should be reinforced.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Cekici A, Kantarci A, Hasturk H, Van Dyke T. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontol*. 2000;64(1):57–80.
2. Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, De Nardin E. Periodontal infections contribute to elevated systemic C-reactive protein level. *J Periodontol*. 2001;72(9):1221–8.
3. Sanz M, Castillo AD, Jepsen S. Periodontitis and cardiovascular diseases: Consensus report. *J Clin Periodontol*. 2020;47(3):268–88.
4. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K. Periodontitis and diabetes: a two-way relationship. *Diabetologia*. 2012;55(1):21–31.
5. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med*. 2005;352(10):1011–34.
6. Madu AJ, Ughasoro MD. Anaemia of Chronic Disease: An In-Depth Review. *Med Princ Pract*. 2017;26(1):1–9.
7. Ž Krmek, Sučić M, Bekić D. Anemia of chronic disease: illness or adaptive mechanism. *Acta Clin Croat*. 2014;53(3):348–54.
8. Wu D, Lin Z, Zhang S, Cao F, Liang D, Zhou X. Decreased Hemoglobin Concentration and Iron Metabolism Disorder in Periodontitis: Systematic Review and Meta-Analysis. *Front Physiol*. 2020;10:1620. doi:10.3389/fphys.2019.01620.
9. Parihar S, Sharma NK, Bhatnagar A, Kishore D, Parihars AV, Rahman F. Comparison of hematological parameters for signs of anemia among participants with and without chronic periodontitis: A cross-sectional study. *J Indian Assoc Public Health Dent*. 2019;17:4–7.
10. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al. Periodontitis: Consensus report of Workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol*. 2018;89(1):S173–82.
11. Lainson PA, Brady PP, Fraleigh CM. Anemia, a systemic cause of periodontal disease? *J Periodontol*. 1968;39(1):35–43.
12. Wilson A, Yu HT, Goodnough LT, Nissenson AR. Prevalence and outcomes of anemia in rheumatoid arthritis: a systematic review of the literature. *Am J Med*. 2004;116(7):50–7.
13. Fuchs D, Hausen A, Reibnegger G, Werner ER, Felmayer GW, Dierich MP, et al. Immune activation and the anaemia associated with chronic inflammatory disorders. *Eur J Haematol*. 1991;46(2):65–70.
14. Faquin WC, Schneider TJ, Goldberg MA. Effect of inflammatory cytokines on hypoxia-induced erythropoietin production. *Blood*. 1992;79(8):1987–94.
15. Seigel EH. Total erythrocyte, leucocyte and differential white cell counts of blood in chronic periodontal disease. *J Dent Res*. 1945;24(1):270.
16. Hutter JW, Velden UVD, Varoufaki A, Huffels RA, Hoek FJ, Loos BG. Lower numbers of erythrocytes and lower levels of hemoglobin in periodontitis patients compared to control subjects. *J Clin Periodontol*. 2001;28(10):930–936.
17. Gokhale SR, Sumanth S, Padhye AM. Evaluation of blood parameters in patients with chronic periodontitis for signs of anemia. *J Periodontol*. 2010;81(8):1202–1208.
18. Naik V, Acharya 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(2):139–182.
19. Gayatri, Ishwary MR, Chaterjee A, Hussain AM, Praveen J, Deepika J. Relationship between periodontitis and anemia - Does it exist? *Indian J Dent Sci*. 2015;7(2):13–16.
20. Aljohani HA. Association between haemoglobin level and severity of chronic periodontitis. *JKAU: Med Sci*;17(1):53–64.
21. Enhos S, Duran I, Erdem S, Buyukbas S. Relationship between iron-deficiency anemia and periodontal status in female patients. *J Periodontol*. 2009;80(11):1750–1755.
22. Patel MD, Shakir QJ, Shetty A. Interrelationship between chronic periodontitis and anemia: A 6-month follow-up study. *J Indian Soc Periodontol*. 2014;18(1):19–25.
23. Siddeshappa ST, Nagdeve S, Yeltiwar RK, Parvez H, Deonani S, Diwan V. Evaluation of various hematological parameters in patients with periodontitis after nonsurgical therapy at different intervals. *J Indian Soc Periodontol*. 2016;20(2):180–183.
24. Siemons L, Klooster T, Vonkeman PM, Riel HEV, Glas PL, Laar CAVD, et al. How age and sex affect the erythrocyte sedimentation rate and C-reactive protein in early rheumatoid arthritis. *BMC Musculoskelet Disord*. 2014;15:368–375.
25. Castro VA, Sampedro MA, Temprano NV, Tuñez C, Rey D, Iglesias CG. Factors influencing erythrocyte sedimentation rate in adults: New evidence for an old test. . *Medicine (Baltimore)*. 2019;98(34):16816. doi:10.1097/MD.0000000000016816.
26. Nemeth E, Ganz T. Anemia of inflammation. *Hematol Oncol Clin North Am*. 2014;28(4):671–81.
27. Anumolu VN, Srikanth A, Paidi K. Evaluation of the relation between anemia and periodontitis by estimation of blood parameters: A cross-sectional study. *J Indian Soc Periodontol*. 2016;20(3):265–72.

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