

Comparison of blood parameters between periodontitis patients and healthy participants: A cross-sectional hematological study

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Abstract

Background: Bacteria and their products involved in periodontitis evoke an immunoinflammatory response in the host tissue. Inflammatory diseases, such as periodontitis, are often not just a local event, but may have systemic ramifications, including elevations in the numbers of circulating leukocytes, acute-phase proteins and oxidative stress markers. It is now emerging that also erythrocytes are affected by chronic inflammatory diseases. This phenomenon, named “anemia of inflammation,” is not caused by marrow deficiencies or other diseases. The present study aimed to assess whether there was any relation between chronic periodontitis and hematological parameters.

Materials and Methods: A total of 80 patients were included in the study and were divided into the healthy and periodontitis groups. Blood sample was obtained from each participant for hematological analysis of leukocytes, erythrocytes, platelets, red blood cell (RBC) distribution width (RDW), mean corpuscular volume (MCV), platelet count and neutrophil–leukocyte ratio (NLR). Further, the values were gathered and subjected to statistical analysis. Unpaired *t*-test was performed to assess the statistical significance between the groups and $P < 0.05$ and < 0.001 were considered to be statistically significant.

Results: Results show statistically significant difference seen in leukocytes, lymphocytes, RDW, MCV, platelet count and NLR which was higher in patients with periodontitis, all other parameters are nonsignificant.

Conclusion: Thus, within limitations, it can be concluded that increased levels of leukocytes, lymphocytes, RDW, MCV, platelet count and NLR depict the inflammatory state and destructive nature of periodontitis.

Keywords: Blood, blood cells, health, periodontitis

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INTRODUCTION

Periodontitis is a multifactorial inflammatory disease affecting the supporting tissues of the teeth caused by specific microbes.^[1] These microorganisms induce an inflammatory reaction in the host tissue for their survival, replication or multiplication that is accomplished by the release of various virulent factors.^[2] For neutralizing this, the body also induces the host response to fight against these pathogens. This host response shows possible mechanism of the relationship between periodontal infection and systemic disorders.^[3]

Sulcular epithelium of gingiva always acts as a protective barrier which prevents the entry of the microorganism and other irritants into the systemic circulation which is one of the life savior mechanisms. Periodontopathogens which accumulate in the sulcus due to plaque and calculus deposits cause ulceration of this sulcular epithelium. These ulcerations provide a passage for these bacteria to enter the systemic circulation.^[2] This bacteremia causes low-grade systemic inflammation. Studies such as Kweider *et al.* 1993,^[4] Ebersole *et al.* 1997,^[5] Wakai *et al.* 1999,^[6] Slade *et al.* 2000^[7] and Loos *et al.* 2000^[8] concluded that periodontitis is associated with the elevated levels of the C-reactive protein and white blood cells.^[9] Anemia of chronic disease (ACD) is defined as anemia occurring in the chronic infection, inflammatory condition and neoplastic condition that is not due to marrow deficiencies and the other diseases and occurring despite the presence of adequate iron store and vitamin study reference article and this phenomenon is known as anemia of inflammation (AI). It is mediated by the effect of the proinflammatory cytokines such as interleukin-6 (IL-6) which ultimately leads to reduced erythropoiesis and shortened the erythrocytes life. A study done by the Nibali *et al.* 2019^[10] which shows there is depression in the erythrocytes number in the patients with chronic periodontitis, another study done by the Hutter *et al.* in 2001^[9] which also shows that patients with chronic periodontitis show the sign of anemia. It is now become apparent that erythrocytes also get affected by chronic inflammatory diseases such as periodontitis.

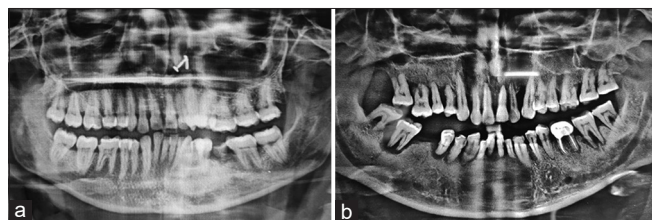


Figure 1: Depicts the orthopantomogram's of the healthy and periodontitis group (a and b)

Thus, the aim of the study was to assess the association between the periodontitis and hematological parameters. The null hypothesis is that there is no association between the leukocytes count, erythrocytes count and platelet count and periodontitis.

MATERIALS AND METHODS

Sample size calculation

Based on G*Power software version 3.1.9.7, (Heinrich-Heine-University, Germany), when the power of the study was kept as 80%, effect size of 0.6 and alpha error value as 5%, the sample size required for conduction of the study was 72. For better analysis of results, the sample size of 80 patients was considered in the present study.

Study design and patient recruitment

The present study was a cross-sectional hematological study. Initially, 120 patients were screened, and finally, a total of 80 participants were selected in the study, 40 patients were eliminated as they did not meet the inclusion criteria and not interested to take part in the study. The present study participants were recruited from the outpatient Department of Periodontics, Institute of Dental Sciences, Bareilly, Uttar Pradesh. After the obtainment of ethical clearance from the institutional ethical committee and informed consent from participants, the study was conducted in accordance with the Helsinki Declaration of 1975 modified in 2000. Patients under the periodontitis group (21 males and 19 females) had a mean age of 41.8 ± 10.28 years and the healthy control group (19 males and 21 females) had a mean age of 36.93 ± 8.24 years [Table 1].

Table 1: depicts the mean age and gender wise distribution along with statistical significance among both the groups.

Variables	Mean \pm SD		P
	Periodontitis (n=40)(%)	Healthy Periodontium (n=40) (%)	
AGE (in years)	41.8 \pm 10.28	36.93 \pm 8.24	0.022*
Sex			
Male	21 (52.5%)	19 (47.5%)	0.654#
Female	19 (47.5%)	21 (52.5%)	

* $P < 0.05$ indicates statistical significance; # indicates Non-Significant; n- number of study participants; % - frequency percentages.

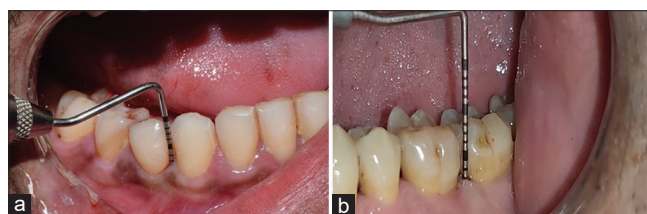


Figure 2: Depicts the pocket depths of periodontitis (a) and healthy group (b)

Inclusion and exclusion criteria

Inclusion criteria for the periodontitis patients were the presence of the periodontal pocket of ≥ 6 mm on at least one site according to recent 2017 classification of periodontal disease by the American Academy of Periodontology,^[11] with radiographic evidence of bone loss on an orthopantomogram [Figure 1], the absence of any systemic disease and no history of periodontitis treatment within 6 months. Further, healthy patients were included by the absence of the periodontal pocket and patients should be systemically healthy. Exclusion criteria for all the patients were known systemic diseases (cardiovascular, respiratory, renal, malignancy, etc.), history and/or the presence of any other infections, systemic antibiotic treatment in the preceding 3 months, long-term treatment with any medication suspected to affect the periodontium (e.g., nonsteroidal anti-inflammatory drugs), patients who are smoker, pregnant or lactating females and < 20 teeth present.

Clinical parameters

Probing pocket depth measurements were done by an individual examiner (RS) using the University of North Carolina Probe (UNC) 15 probe (Hu-Friedy, USA) [Figure 2]. Before final measurements of the study, 10 periodontitis patients whose data were not included in the present study were considered for probing and measurements performed 72 h apart and values were considered for 90% agreement if the difference range is within ± 1 mm.^[12]

Blood parameters and sampling

Blood sampling and analysis were done in the Department of Oral and Maxillofacial Pathology, Institute of Dental Sciences, Bareilly, Uttar Pradesh. For both the groups, blood samples were obtained from each patient through venipuncture of the right arm during the examination visit. The samples were collected and analyzed for the number of leukocytes, differential leukocyte count, red blood cells (RBC), hemoglobin (Hb%), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), MCH concentration (MCHC), neutrophil-lymphocytes ratio (NLR) (NLR = total neutrophil count/absolute lymphocyte count), RBC distribution width (RDW), mean platelet volume (MPV), number of platelets.

Statistical analysis

After gathering the data of the hematological parameters of the patients that were included in the study, the entire data were tabulated in the Excel spreadsheet. Unpaired *t*-test was used to check the statistical significance. The mean standard deviation was calculated for all the parameters in both the groups and *P* was kept as < 0.05 as significant and < 0.001 as highly significant

RESULTS

The mean age and gender-wise distribution of both the groups were depicted as demographic data where the significant difference ($P = 0.022$) was obtained regarding age and values were nonsignificant ($P = 0.654$) regarding gender in both the groups [Table 1]. Regarding the mean total leukocyte counts, mean lymphocyte percentages and mean MCV statistical significance difference ($P = 0.03$, $P = 0.027$ and $P = 0.019$) were reported for the chronic periodontitis group than the health group. Further, values were high statistically significant for RDW% ($P < 0.001$) and MPV ($P < 0.001$), while for the remaining parameters, there was no statistical significance recorded ($P > 0.05$) when both the groups were compared [Table 2].

DISCUSSION

Anemia is considered one of the most common global public health problems. It usually occurs in about 25% of the population globally. Iron deficiency is one of the most common causes of anemia^[13] and periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth causing progressive attachment and bone loss.^[14] It was shown that long-standing chronic inflammatory disease can lead to anemia, and this anemia is known as ACD.^[15,16] Previous studies by Hutter *et al.* 2001 and Gokhale *et al.* 2010^[2,9] suggested that anemia is an important factor in the etiopathogenesis of periodontitis. As much as the number of studies was not performed for confirmation, the present study was conducted to rule the

Table 2: depicts the statistical comparison of various haematological parameters with periodontitis and healthy groups.

Haematological Parameters	Mean \pm SD		t	P
	Periodontitis (n=40)	Healthy (n=40)		
LEUKOCYTES/CUMM	8090 \pm 1702.46	7397.5 \pm 1014.38	2.210	0.030*
NEUTROPHILS%	68.63 \pm 7.95	63.43 \pm 7.57	2.995	0.004**
LYMPHOCYTES %	34 \pm 8.32	30.23 \pm 6.6	2.248	0.027*
MONOCYTES%	1.38 \pm 1.5	0.95 \pm 1.55	1.247	0.216
EOSINOPHILS%	3.83 \pm 1.89	3.95 \pm 1.58	-0.320	0.750
BASOPHILS%	0.05 \pm 0.22	0 \pm 0	1.433	0.156
RBC MILLION/CUMM	4.4 \pm 0.88	4.63 \pm 0.79	-1.186	0.239
HB%	13.51 \pm 1.58	13.58 \pm 1.49	-0.211	0.833
HCT%	39.19 \pm 5.38	41.08 \pm 4.59	-1.698	0.094
MCV fL	86.26 \pm 10.38	81.6 \pm 6.57	2.401	0.019*
MCH pq	32.29 \pm 3.72	32.98 \pm 4.32	-0.769	0.444
MCHC q/dl	34.04 \pm 2.64	34.07 \pm 3.36	-0.488	0.627
NLR ratio	2.48 \pm 1.17	2.02 \pm 0.5	2.24	0.013*
RDW %	50.31 \pm 8.93	40.52 \pm 5.48	5.914	< 0.001 **
MPV fL	12.7 \pm 1.41	12.74 \pm 1.2	-0.128	0.898
PLATELET NO. lacs/mm ³	2.91 \pm 0.95	2.03 \pm 0.66	4.815	< 0.001 **

* $P < 0.05$ (significant), ** $P < 0.001$ (highly significant)

possible relation regarding the levels of blood parameters in periodontitis patients and healthy periodontium.

In the present study, smokers were excluded because smoking is considered the cofounding factor for the development of periodontitis. Smoking affects the immune system and microflora of the oral cavity causing deeper probing depth and greater attachment loss and bone loss.^[17,18] Nicotine also alters the neutrophils function such as phagocytosis, superoxide production and protease inhibitor production. Smoking also affects erythrocytes count and other blood parameters. A study by Erdemir *et al.*^[19] also supported the statement where smokers with chronic periodontitis have a lower number of erythrocytes count, lower value of Hb and lower HCT compared to nonsmokers with chronic periodontitis.

In the present study, leukocyte count is more in the periodontitis group than in the healthy group which is in accordance with the previous study by Kwon *et al.* 2016 demonstrating increased leukocyte count in the patient with chronic periodontitis, especially in the number of the neutrophils and lymphocytes. This increase in the number is the typical response of the periodontitis which occurs as a part of the inflammatory cascade, initiated by the accumulation of the periodontopathogens which are present subgingivally.^[20]

The result of the present study regarding decreased RBC, Hb%, MCH, MCHC and HCT was in accordance with the previous study done by Siebert *et al.* 2015^[21] where they have concluded that which there is decrease in hematological parameters in periodontitis patients. Lower value of the HCT can be attributed to the lower number of erythrocytes. This depressed erythropoiesis by systemically circulating proinflammatory cytokines was resulted from a local chronic inflammatory process. Thus, proinflammatory cytokines were thought to act as mediators in suppressing erythropoiesis from the bone marrow leading to anemia. Cytokines such as IL-1 alpha, IL-6 and tumor necrosis factor-alpha ((TNF-a) have been related to the suppression of erythropoiesis. TNF-a administration to animals by intermittent injections or implantation of TNF-a-producing cells resulted in the development of anemia. Thus, the above results were even supported by previous studies^[2,9] where the relation between periodontitis and AI has been demonstrated and reported a reduced RBC count, HB%, MCH, MCHC, HCT and packed cell volume in patients with periodontitis.

In the present study, although the number of RBC decreased in periodontitis patients, the results do not show

any statistical difference. Similar result was obtained in the level of Hb and this was attributed due to the lower number of RBCs or due to the iron-binding protein hepcidin which may be related to the reduced amount of circulating Hb. Hepcidin increases its activity in the bloodstream due to inflammatory stimulus leading to the internalization and closure of ferroportin channels, found on the surface of cells, preventing the iron output to the plasma and resulting in the reduction of Hb formation.^[22] MCH and MCHC values were slightly lower in periodontitis patients but did not reach statistical significance. Generally, it is known that depressed MCV values show microcytic anemia and increased MCV show macrocytic anemia, while in the present study, the value of MCV is more in the periodontitis patients but remains in the normal range.

In the present study, the platelet count and mean platelet values were higher in patients with periodontitis when compared to the healthy individuals and the results are in accordance to the previous study by Romandini *et al.*, 2018^[23] where they have concluded that there was increase in the platelet count in the patient with periodontitis. It has been demonstrated that dental plaque bacteria, including the periodontal pathogen induces platelet activation and aggregation and the main etiology of the periodontitis is the periodontal pathogens this will cause the activation of the platelets.^[24] In the present study, the NLR was found to be higher in the chronic periodontitis patient and this was in accordance with the study of Rejec *et al.* 2017,^[25] in which there was also increase in the NLR in the patient suffering from chronic periodontitis. Increase NLR shows the initial innate mechanism which triggers the adaptive immune response and results in the destruction of the periodontal tissue.^[26]

The results of the present study confirm the relation between periodontitis and anemia. However, the following limitations of the present study need to be fulfilled in future studies for the establishment of better outcomes. First, the lesser sample size as the larger samples size gives more significant results or the clear relation between periodontitis and blood parameters. Second, the comparison of the pretherapeutic and the posttherapeutic changes in the blood parameters of the patients suffering from periodontitis, this would add in our understanding about the changes in the blood parameters in periodontal disease.

CONCLUSION

Thus, within limitations, it can be concluded that chronic inflammatory diseases such as periodontitis may cause anemia as the patients suffering from periodontitis having

a lower number of erythrocytes, Hb, HCT value, MCH and MCHC. The low-grade inflammation may cause marked leukocytosis due to increase in the number of leukocytes and neutrophils. However, future studies are necessary to draw a definite conclusion.

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Conflicts of interest

There are no conflicts of interest.

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