Original Article

Evaluation of Haematological Changes in Patients with Chronic Periodontitis & Gingivitis in Comparison to Healthy Controls - A Clinical Study

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Abstract:

Background: The role of periodontal diseases in influencing the systemic diseases such as diabetes mellitus, cardiovascular diseases and pulmonary diseases has been reported. ***Aim***: The current study was carried out to evaluate the differences in levels of the components of peripheral blood in patients with chronic periodontitis and gingivitis and compare it with healthy controls. ***Methods and Materials*:** A total of 62 patients, 31 male and 31 female, were divided into 3 groups namely; healthy controls (10 male, 8 female), gingivitis (10 male, 9 female) and chronic periodontitis (11 male, 14 female) and were evaluated for haemoglobin percentage, red blood cell count, haematocrit, erythrocyte sedimentation rate, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total leukocyte count, neutrophil, lymphocyte, monocyte and eosinophil percentages. ***Statistical tools used:*** Two factor ANOVA and Scheffe's Post Hoc test ***Results:*** The mean red blood cell counts and haematocrit were lower in female patients with periodontitis as compared to the healthy female patients and females patients with gingivitis. The haemoglobin percentage, haematocrit and mean corpuscular haemoglobin showed significant differences between the gingivitis and periodontitis groups ($p<0.05$). Total leukocyte counts, lymphocyte and monocyte percentage counts were significantly different between the healthy and periodontitis groups. The neutrophil counts were significantly different between healthy and gingivitis groups ($p<0.05$).

Conclusion: The results indicate that chronic periodontitis may alter the levels of the components of the peripheral blood.

Keywords: Chronic Periodontitis, Haemoglobin, Erythrocyte, Haematocrit, Leukocyte

Introduction:

The accumulation of plaque on the tooth surface, along the gingival margin initiates an inflammatory reaction in the host tissue. This inflammatory reaction may get localized to an area coronal to the junctional epithelium and is known as gingivitis. In some patients and at some sites the phase of gingivitis is followed by an extension of this inflammatory reaction into the underlying supporting structures resulting in the loss of connective tissue attachment and supporting alveolar bone.[1] This destruction is caused either by direct toxic effect of a group of gram-negative bacteria- mainly *Porphyromonas gingivalis, Actinobacillus actinomycetumcomitans, Tanerella forsythenesis*[2] or by exaggerated host response to the bacteria mounted by the polymorphonuclear leukocytes (PMNs) initially and later by the cells of the reticulo-endothelial system, i.e. monocytes, macrophages[3] and a combination of both.

The exaggerated host response to these bacterial products results in elevated levels of pro-inflammatory cytokines i.e. IL-1, IL-2, IL-6, TNF- , IFN- and also the release of high amounts of acute phase reactants like C-reactive protein[4-6]. The elevated levels of these cytokines and acute phase reactants in the serum lead to conditions that can be addressed as systemic inflammation. The role of periodontal diseases as a possible risk factor for systemic diseases and conditions has heralded the dawn of periodontal medicine as an emerging branch of Periodontology.[7] The influence of elevated levels of pro-inflammatory cytokines in chronic periodontitis on the diabetic status[8], pre term low birth weight (PTLBW)[9] and coronary heart disease[10] have been reported.

Hutter et al[11] found lower levels of erythrocytes and heamoglobin in caucasian patients with periodontitis. They also found that the ESR was elevated, as compared to healthy individuals.

The persistently high levels of the pro-inflammatory cytokines in chronic periodontitis may predispose an individual to develop anemia, referred to as anemia of chronic disease (ACD). ACD is described in the literature as one of the common forms of anemia caused by iron deficiency secondary to immune activation. The immune activation leads to a series of changes which are started by the pro-inflammatory cytokines and cells of the reticulo-endothelial system (RES) which results in the changes associated with iron homeostasis, erythroid progenitor proliferation, production of erythropoetin and life span of RBCs, all of which contribute to its pathogenesis.[12-14] The high levels of cytokines may also influence other blood parameters. This study was conducted to evaluate the differences in the levels components of the peripheral blood in patients with chronic periodontitis and gingivitis and compare with healthy subjects and also evaluate if there were any anemic changes.

Materials and Method:

The present cross-sectional study was carried out on South Asian subjects. A total of 62 subjects (31 male and 31 female),

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referred to the Dept. of Periodontics, SDM College of Dental Sciences and Hospital, Dharwad were selected. All the patients included in the study were duly informed and written consent was obtained. The study was approved by the ethical committee of the SDM College of Dental Sciences and Hospital, Dharwad. All the subjects were enrolled during 2004-2005.

The 62 subjects were then divided into 3 groups:

1. Healthy Group: Eighteen Healthy subjects (10 male and 8 female) were categorized as controls, who did not display any clinical signs of gingival disease.
2. Gingivitis Group: Nineteen subjects (10 male and 9 female) is displayed signs of gingivitis with Loe and Silness [15] Gingival index score of 2.
3. Chronic Periodontitis Group: Twenty five patients (11 male and 14 female) who displayed probing pocket depths and loss of attachment with >50% of bone loss in at least 8 teeth of the permanent dentition.

The exclusion criteria included- history of any periodontal therapy in the past 6 months, antibiotic intake in the past six months, pregnant women, lactating mothers, patients undergoing any medical treatment and smokers, patients on any medications, patients with any systemic conditions such as diabetes mellitus and cancers.

Collection of Data:

The peripheral blood samples were collected by veni puncture in the antecubital fossa at the clinico-pathological laboratory of the institution. All the reagents were purchased and the samples were immediately analyzed for the following: hemoglobin percentage (Hb%, gm%) by cyan meth hemoglobin method and analyzed by automated analyzer , red blood cell counts (million cells/ cubic mm) were counted on modified Neubauers chamber, hematocrit by Wintrobes's method, erythrocyte sedimentation rate by Westergren method (ESR mm/hr) . Mean corpuscular volume (MCV 10-15L), mean corpuscular hemoglobin (MCH 10-3 grams), mean corpuscular hemoglobin concentration (MCHC %) were calculated. Total leukocyte count (TLC, cells/ cubic mm) was done on modified Neubauers chamber. The differential count was done to find out the neutrophil (N %), lymphocyte (L %), monocyte (M %) and eosinophil (E %) percentages.

Statistics:

The data was analyzed using commercially available software program. Two-factor ANOVA with age as a co-variate was done to evaluate effects between the sexes and between the groups for all the variables and also to find the sex and group interactions. If any significant effects were found between the sexes, between the groups or sex and groups interactions they were further analyzed by Scheffe's post hoc test. The significance was set at P- value of $p < 0.05$ for all analyses.

Results:

The data from the male and the female subjects was analyzed for all the variables. The data and the results of the two-factor ANOVA and Scheffe's post hoc tests for interaction between the sex and group and between the group differences are described in Table 1, 2 and 3 respectively. The mean age of the female subjects was 26.6±2.7 years for the healthy group, 29.9 ±11.5 years in the gingivitis group and 36.8 ±9.1 years in the periodontitis group. In the male subjects the corresponding data were 29.2± 4.4years for the healthy, 32.6 ±9.3 years for the gingivitis and 42.4 ±11.5 years for the periodontitis groups respectively. The mean values for the RBC counts and haematocrit were lower in the female patients with periodontitis as compared to the healthy female subjects and female patients with gingivitis. The mean ESR and TLC values in the female and male patients with periodontitis were higher as compared to the control and gingivitis group of the respective sexes.

The haemoglobin percentage showed significant difference between the sexes and between the groups (Table 1), but did not show any significant interactions between the sexes and the groups, while the RBC counts showed a significant difference between the sexes but not between the groups; however there was a significant interaction between the sexes and groups (Table 1).

The haematocrit values showed significant differences between the sexes, between the groups and also showed significant interaction between the sexes and the groups.

MCH and ESR values showed significant differences between the sexes but not between the groups, there was also no group and sex interaction. MCHC, TLC, L% and M% counts were significant between the groups but not between the sexes.

Scheffe's post hoc analysis was carried out for RBC counts and haematocrit values for sex and group interactions (Table 2). The RBC data displayed a significant difference between female patients with gingivitis and female patients with periodontitis. The haematocrit data showed a significant difference between the healthy female subjects and females with periodontitis.

The Scheffe's post hoc analysis for between the groups' comparisons was carried out to find out the differences

Table 1: Two way ANOVA with (age as a covariate) for all the parameters.

Variable		Female Patients			Male Patients			p-values		
	Summary	Healthy	Gingivitis	Periodontitis	Healthy	Gingivitis	Periodontitis	Sex	Group	S x G
Hb (gm %)	Means	12.46	13.18	11.83	14.55	14.77	14.39	0.00*	0.03*	0.16
	S.D.	0.84	0.72 1.05	0.52	1.12	0.69				
RBC (1012/L)	Means	4.27	4.36 3.89	4.92	4.87	4.90	0.00*	0.52	0.01*	
	S.D.	0.27	0.24 0.37	0.19	0.39	0.40				
Hematocrit (%)	Means	41.87	40.00	36.64	43.90	45.40	43.63	0.00*	0.03*	0.00*
	S.D.	2.35	2.64 2.37	1.72	3.56	2.54				
MCV(10-15/L)	Means	98.27	91.60	94.51	89.26	93.31	89.34	0.12	0.44	0.54
	S.D.	8.06	3.14 5.60	2.74	3.94	5.42				
MCH(10-3 gm)	Means	29.22	30.20	30.43	29.58	30.34	29.47	0.01*	0.25	0.63
	S.D.	2.20	0.37 0.74	0.85	0.70	1.73				
MCHC (%)	Means	29.88	33.01	32.27	33.15	32.54	33.00	0.96	0.03*	0.16
	S.D.	3.04	1.24 1.39	0.93	0.76	0.72				
ESR (mm/hr)	Means	5.37	7.77 14.42	4.60	5.80	7.72	0.03*	0.32	0.19	
	S.D.	2.82	3.83 12.15	1.95	3.93	4.79				
TLC	Means	7831.25	8716.66	9214.28	7650	8185	8745.45	0.07	0.03	*0.76
(Cells/ mm3)	S.D.	260.40	1051.19	645.52	1370.52	1404.96	731.25			
N (%)	Means	65.50	60.77	59.78	62.60	60.70	60.00	0.37	0.73	0.79
	S.D.	0.53	7.42 7.46	6.86	6.20	5.310				
L (%)	Means	31.62	37.00	36.64	28.30	38.00	36.18	0.63	0.00*	0.60
	S.D.	1.30	7.76 7.63	8.48	6.41	4.89				
E (%)	Means	1.87	1.88 2.14	1.90	1.90	2.18	0.72	0.17	0.97	
	S.D.	0.64	0.78 0.94	0.56	0.73	0.60				
M (%)	Means	1.00	1.11 1.28	1.10	1.00	1.54	0.37	0.00*	0.44	
	S.D.	0.75	0.33 0.61	0.56	0.00	0.52				

**Significance = significance at p-value p<0.05*

between the healthy, gingivitis and periodontitis groups (Table 3).

The hemoglobin percentage and MCHC showed significant difference between gingivitis and periodontitis groups. Haematocrit values showed significant difference between healthy, gingivitis and periodontitis patients.

TLC and monocyte percentage counts were different between the healthy and periodontitis patients.

The neutrophil percentage counts did not show any significance, whereas the lymphocyte percentage counts were significantly different between the healthy, gingivitis and periodontitis patients.

Discussion:

The two-way relationship between periodontal diseases and systemic diseases is being studied and role of periodontal diseases as a risk for systemic diseases has been reported.[7-10] This association has been related to elevated levels of pro-inflammatory cytokines in the local gingival tissues as well as in the systemic circulation.[3] The increased levels of pro-inflammatory cytokines alters the iron homeostasis, erythroid progenitor proliferation, production of erythropoietin and the life span of RBCs- all of which contribute to development of anemia of chronic disease.[12-14] Hutter et al found lowered levels of erythrocytes, lowered hemoglobin and increased ESR in Caucasian subjects with periodontitis.[11] However studies by Wakai report any significant relation between the CPITN scores and hemoglobin levels.[16] In the present study we evaluated the subjects belonging to South Asia. All the subjects were examined for levels of the components of peripheral blood namely hemoglobin percentage, RBC count, haematocrit, MCH, MCHC, ESR, TLC, lymphocyte, neutrophil, monocyte and eosinophil percentage counts. Our study did not include smokers as it has been found that smoking influences the levels of the components of the peripheral blood irrespective of periodontal status.[11] In the current study the mean values of the Hb %, RBC counts, hematocrit, MCH, ESR were lower in healthy female subjects than healthy male subjects, this can be attributed to the physiological differences between the two sexes.[17, 18]

The Hb% showed significant difference between the gingivitis and periodontitis patients (Table 3). This difference can be attributed to the altered iron homeostasis secondary to the presence of chronic inflammation in the body in the form of periodontitis. It has been hypothesized that there is increased uptake and retention of iron by the cells of the RES in chronic inflammation thus reducing the availability of iron for erythroid progenitor cells.[14]

Lowered RBC counts in the female patients with periodontitis patients as compared to females with gingivitis (Table 2) can probably be related to reduction in the erythroid progenitors[13,14], due to the presence of chronic inflammation in the form of periodontitis. It has been observed that elevated levels of pro-inflammatory cytokines namely IL-1, TNF- and IFN- due to chronic inflammation reduces the number erythroid progenitors.[13, 14]

We found that the hematocrit value in the female patients with periodontitis was less compared to the healthy females (Table 2). This finding is similar to those reported by Hutter et al[11]. The hematocrit values are dependent on the RBC counts and MCV, if either or both of them are lowered then the hematocrit value decreases. In the current study as the MCV values were not significantly different between the three female groups we can attribute the lowered hematocrit to the lowered RBC counts.[19, 20]

The MCHC values were lower in periodontitis patients as compared to gingivitis patients (Table 3). Weiss and Goodnough attribute the lowering of the MCHC in chronic diseases to lowered saturation of the red cell by hemoglobin secondary to lowered iron homeostasis in the presence of chronic inflammation.[14] The lowering of MCHC can be

Table 2.Scheffe's post hoc test for interaction between the sex and group

Variable	Interaction: Sex x Group	Female x Healthy	Female x Gingivitis	Female x Periodontitis
RBC counts (1012/L)	Mean	4.27	4.36	3.89
	FemalexHealthy	1.00		
	FemalexGingivitis	0.99	1.00	0.04*
	FemalexPeriodontitis	0.20	1.00	
Hematocrit (%)	Mean	41.87	40.00	36.64
	FemalexHealthy	1.00		
	FemalexGingivitis	0.80	1.00	0.11
	FemalexPeriodontitis	0.00*	1.00	

*Significance = significance at p-value p<0.05

attributed to the presence of chronic periodontitis but this fact has to be further evaluated in longitudinal studies.

The TLC was high in periodontitis patients as compared to healthy patients which can be related to the presence of infection in the body. The lymphocyte counts were higher in both gingivitis as well as periodontitis patients as compared to healthy subjects. This can probably be related to the inflammatory state. The elevated monocyte counts in periodontitis patients as compared to healthy patients may be related to chronic inflammation and secondary to overall increase in the total leukocyte count.

In the current study the mean ESR values were found to be higher in the periodontitis patients as compared to healthy and gingivitis group, but were not statistically significant. Hutter et al reported that the ESR was elevated in the periodontitis patients and this was related to elevated cytokine levels in the serum due to the presence of chronic inflammation which influences the rouleaux formation by the RBC's.[11-21] The ESR values showed significant difference between the sexes but there was no difference between the groups.

In our study the age of the healthy patients was on an average younger than the other two groups. While the covarying the age in the analysis did remove the linear effects of age on the data, but systematic differences due to the age per se persist.

Our data indicates that levels of the blood components in female patients are different as compared to males. Female patients with periodontitis have altered levels of blood components as compared to female patients with gingivitis and healthy females. The data from the male subjects does not depict any differences between the groups, this leads to the fact that whether the observed differences in the female subjects are due to the presence of chronic periodontitis or are merely due to the physiologic differences between the sexes[18,19].

Table 3. Scheffe's post hoc test for between the group comparisons.

Variable	Group	Healthy	Gingivitis	Periodontitis
Hemoglobin (gm%)	Mean	13.50	13.97	13.11
	Healthy	1.00		
	Gingivitis	0.25	1.00	0.01*
	Periodontitis	0.34	1.0000	
Hematocrit (%)	Mean	42.88	42.70	40.1396
	Healthy	1.00		
	Gingivitis	0.97	1.00	0.01*
	Periodontitis	0.00*	1.00	
MCHC (%)	Mean	32.91	32.87	31.75
	Healthy	1.00		
	Gingivitis	0.94	1.00	0.04*
	Periodontitis	0.03	1.00	
TLC (Cells/ cumm)	Mean	7740.62	8450.83	8979.87
	Healthy	1.00		
	Gingivitis	0.09	1.00	0.21
	Periodontitis	0.00*	1.00	
L(%)	Mean	29.96	37.50	36.41
	Healthy	1.00		
	Gingivitis	0.00*	1.00	0.86
	Periodontitis	0.01*	1.00	
M(%)	Mean	1.05	1.05	1.41
	Healthy	1.00		
	Gingivitis	0.99	1.00	0.08
	Periodontitis	0.04*	1.00	

**Significance = significance at p-value p<0.05*

Further studies will have to be conducted to ascertain the influence of chronic periodontitis on the components of the peripheral blood and whether the relation is causal or casual has to be evaluated.

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