Original Article

Evaluation of Non-surgical Treatment in Chronic Periodontitis Patients on Blood Parameters

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Abstract

Introduction: Anemia of chronic disease (ACD) is the second most common form of anemia after iron deficiency anemia. This type of anemia occurs in cases of chronic infections, inflammatory conditions, or neoplastic disorders and even in presence of enough iron and required vitamins. Some previous studies have suggested that periodontitis, as a chronic disease, is likely to be associated with this type of anemia. The aim of this study is to investigate the possible effect of non-surgical periodontal therapy on improvement of blood parameters. **Methods:** This study was performed on 36 male patients with chronic moderate or severe periodontitis (divided into case and control groups) and 18 men with healthy periodontium. Blood samples such as hematocrit, hemoglobin, MCH, MCHC, MCV and Ferritin were collected from participants. Then periodontal treatment was started for case group. **Results:** In the case group, there was a significant increase in hematocrit, hemoglobin, MCH, MCHC and MCV after 8 weeks of treatment and there was no significant decrease of Ferritin. No significant differences in blood parameters were observed in periodontally healthy and control groups. **Conclusion:** According to significant differences in some mentioned blood parameters after non-surgical treatment, it seems that periodontal assessment and subsequent therapy can be recommendable as an adjunctive part in overall treatment plan of anemic patients**.**

Keywords: Anemia, Blood Parameters, Chronic Periodontitis, Periodontal Treatment

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Introduction

Periodontitis is a common disease (1) involving supporting tissues of teeth through a chronic infection (2). Previous epidemiological studies suggested the relationship between periodontal diseases and systemic conditions including cardiovascular problems (3), cerebrovascular diseases (3), atherosclerosis (4), preterm birth with low birth weight (5) and Diabetes mellitus (6). Recently few studies have also reported the relationship between anemia and periodontal disease (7,8). Pre-inflammatory cytokines play a significant role in progress of periodontal disease (9). Cytokines bind to receptors on various target cells and cause transmission of intercellular signals. Low levels of these proteins affect different type of cells. Cytokines binding to cell surface receptors initiate intercellular changes that produce a group of materials and cause cell behavior changes. These events eventually cause increase in cytokine secretion. Due to the positive feedback, these continuous changes cause inflammation. Cytokines are produced by various inflammatory cells such as neutrophils, macrophages, lymphocytes and periodontal

cells including epithelial cells and fibroblasts. Cytokines are in conjunction with each other and usually do no act alone. Cytokines can induce biological changes or tissue damages such as destruction of connective tissue and alveolar bones. These cytokines may be also effective in creating anemia by preventing proliferation and differentiation of erythrocyte precursors (10) and changing the metabolism of iron (11). In addition, they can suppress release of erythropoietin hormone from kidneys causing anemia (12).

Routine laboratory test for anemia is as follows: Complete blood count (CBC) including Hb, HCT and erythrocyte indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and Ferritin.

Anemia of chronic disease (ACD) is one of the most common types of anemia observed in patients with inflammation, infection and tumors. Such anemia occurs despite presence of sufficient iron reservoirs and normal function of bone marrow (13). ACD is typically recognized as a normocytic normochromic anemia. Patients with this type of anemia have a certain pattern of iron distribution in which, serum iron (SI) and Total Iron-Binding Capacity (TIBC) are low or normal while the level of Ferritin is high or normal. (14) Such changes occur due to presence of Hepcidin regulating iron level. Hepcidin is a hormone produced by liver in response to inflammatory cytokines such as IL-6 or other inflammatory cytokines that are high in blood circulation in periodontitis (15). Briefly, periodontitis may lead to a systemic chronic inflammation that may reduce RBCs and hemoglobin in patient's blood.

In a study in 2012, red blood cell count (RBC), hemoglobin and hematocrit were significantly lower among patients with generalized chronic periodontitis. However, no significant difference was observed in MCV, MCH and MCHC (16). In another study in 2016, RBC, hemoglobin and PCV in periodontal patients was significantly lower than the control group (8).

Treatment of anemia is important due to its reducing effect on blood supply of periodontal tissues along with other systemic side effects. There are some controversies about the results of previous studies on the relationship between presence of periodontitis and reduction of blood indices. On the other hand, there are only few clinical studies about effect of Periodontal treatment on blood indices. The present study designated to determine possible role of periodontal therapy in improvement of blood parameters.

Materials and Methods

The clinical trial entry number of this study was IRCT20151013024509N3 and it was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences.

The patients participating in this study completed and signed the consent form. In addition, no fee was taken for laboratory tests or periodontal treatment. In the case of having abnormal blood indices, a consultation was made with our hematologist for probable further interventions.

Because of ethical considerations, selection of patients with certain anemia level and then subsequent delay in their treatment for 8 weeks was not possible. So, we had to choose patients with normal ranges of blood parameters whose indices should have been improved according to the opinion of our hematologist.

Thirty-six 25-50 years old males with moderate to severe chronic periodontitis referred to hematology clinic of Shahid Sadoughi Hospital were selected (except for periodontitis, they were perfectly healthy with no systemic diseases). 18 out of these 36 subjects received non-surgical periodontal treatment immediately (case group) while the treatment began for the remaining 18 patients after eight weeks (control group). It should be mentioned that means of age and periodontal and hematological indices of both case and control groups were matched before beginning of the study by overall matching. Other 18 males without any periodontal disease were selected as periodontally healthy group. To minimize bias of probable random changes in hematological indices during the 8 weeks of study, all efforts were made for matching age and similar blood parameters to between groups even without any specified intervention. The inclusion and exclusion criteria are summarized in Table I.

Table I. Inclusion criteria

Male patients (25-50 years)
No systemic disease
No history of hospitalization and donating blood
No history of receiving
No periodontal treatment in the past three months
No use of cigarettes, alcohol and tobacco, serum ferritin levels greater than 30 ng / ml
No use of the following drugs: warfarin, Plavix, lithium , Aspirin, corticosteroid drugs, heparin, phenytoin, Methyldopa, Nonsteroidal anti-inflammatory drugs (NSAIDs), factors causing coagulation, blood pressure-lowering drugs

First, an informed consent was received from all patients and the questionnaire was completed. After selection of samples, periodontal indices including BOP, PPD, and CAL and PI were recorded by a post-graduate student of periodontology.

All three groups were instructed about appropriate oral hygiene instruction including brushing and flossing using a dental model. The first phase of periodontal treatment (scaling and root planning and polishing by ultrasonic device (Guilin Woodpecker Medical Instrument, China) was performed by the same post-graduate student of periodontology in the case group. It should be noted that patients with severe periodontitis were followed up at the end of the study to evaluate for any further surgical treatment need. Four weeks later, the case group was called for checking the adequacy of periodontal treatment and if necessary, supplementary periodontal treatment was performed. In case of no appropriate oral health, oral hygiene instructions were re-enforced. After eight weeks from the beginning of the study, all periodontal indices were re-measured by another post-graduate student.

Two cc of venous blood was taken from all participants to evaluate blood indices such as hemoglobin, hematocrit, MCV, MCH, MCHC and serum Ferritin after periodontal examinations in the follow up stage (eight weeks later). CBC tests were performed using KX21 cell counter (Sysmex® Automated Hematology Analyzer KX-21) and the Ferritin test was performed by Electrochemiluminescence e411 device. The results were analyzed using SPSS 23 software by independent and paired t-testand ANOVA test. The level of statistical significance was assigned at 0.05.

Results

Eighteen patients with healthy periodontium (group A) were selected. Half of the patients with chronic periodontitis(18 out of 36) went under periodontal treatment (group B) while the other half were treated after the end of the study (group C). The mean age of patients participating in all three groups was not significantly different. (P-value = 0.801)

The mean PPD, CAL, BI and PI in each of three groups at the beginning of the study and eight weeks later is presented in Table II. The mean PPD (P-value = 0.859) and PI (P-value =0.093) in the group A were not statistically significant at the beginning and eight weeks after the study. However, the mean PI (P-value =0.012) indicated a significant difference.

Table II: Comparison of mean periodontal indices of each group at the beginning of the study and eight weeks later

Periodontal Index / Group		PPD (mm)	CAL (mm)	BI (%)	PI (%)
Healthy (A)	beginning of the study	2.17±0.20	0	2.60±4.21	23.69±8.05
	eight weeks later	2.14±0.13	0	2.70±5.43	20.89±8.00
	P-value	0.859	0.092	0.093	0.012 *
Case (B)	beginning of the study	4.89±0.62	5.14±0.78	61.09±10.20	62.91±8.95
	eight weeks later	2.98±0.32	3.27±0.47	18.06±5.99	19.75±3.89
	P-value	<0.001 *	<0.001 *	<0.001 *	<0.001 *
Control (C)	beginning of the study	4.92±0.52	5.27±0.72	61.80±8.48	62.29±9.52
	eight weeks later	4.91±0.51	5.22±0.72	56.50±16.15	58.09±9.09
	P-value	0.644	0.163	0.407	0.004 *

* Significant with more than 95% confidence (P value <0.05) based on Paired T-test

In group B, mean PI, PPD, BI and CAL indices at the end of the study was significantly less than the beginning of the study (P-value <0.001).

The mean BI, PPD and CAL indices in group C at the end of the study was less than the beginning of the study while no statistically significant difference was observed (P-values were 0.407, 0.644, 0.163). Meanwhile, the mean PI decreased and it was statistically significant (P-value = 0.006).

There was a significant difference in terms of BOP, CAL, and probing depth (P-value = 0.001) and PI (P-value= 0.000) between group B and C at the end of study. (Table III)

Table III. Comparison of mean periodontal indices in the case (B) and control (C) groups at the beginning of the study and eight weeks later

Periodontal Index / Group		PPD (mm)	CAL (mm)	BI (%)	PI (%)
beginning of the study	Case (B)	4.89±0.62	5.14±0.78	61.09±10.20	62.91±8.95
	Control (C)	4.92±0.52	5.27±0.72	61.80±8.48	62.29±9.52
	P –value	0.879	1.000	0.957	0.976
eight weeks later	Case (B)	2.98±0.32	3.27±0.47	18.06±5.99	19.75±3.89
	Control (C)	4.91±0.51	5.22±0.72	56.50±16.15	58.09±9.09
	P- value	0.001*	0.001*	0.001*	0.001 *

* Significant with more than 95% confidence (P- value <0.05) based on ANOVA test

In group B, mean values of hematocrit, hemoglobin, MCH, MCHC, and MCV indicated a statistically significant increase at the beginning of the study and eight weeks later and it was also clinically significant according to the co-hematologist. Furthermore, mean ferritin at the end of the study was slightly lower than the beginning which was not statistically significant. No significant difference in blood parameters was seen in group C. After eight weeks, no significant changes in blood parameters was seen in group A (Table IV)

Changes of mean blood indices in groups B and C were also compared in the present study (Table V).

Table IV: Comparison of mean blood indices of each group at the beginning of the study and eight weeks later

Blood parameter / Group		Hematocrit (%)	Hemoglobin (g/dl)	Ferritin (ng/ml)	MCH (pg)	MCHC (g/dl)	MCV (fl)
Healthy(A)	beginning	44.03±1.48	15.08±1.06	134.53±55.83	29.82±1.41	32.67±1.99	83.18±3.45
	eight weeks later	43.94±1.92	15.00±0.83	147.03±71.85	29.28±1.30	33.00±1.86	83.63±2.87
	P-value	0.808	0.504	0.580	0.110	0.289	0.435
Case (B)	beginning	43.13±1.53	14.84±0.58	135.88±59.51	29.36±1.27	32.92±2.02	83.62±3.26
	eight weeks later	45.85±1.67	15.87±0.57	124.68±37.25	30.91±1.59	34.16±2.11	85.91±3.09
	P-value	<0.001 *	<0.001 *	0.076	0.004 *	0.038 *	0.023 *
Control (C)	beginning	43.49±1.27	14.66±0.73	133.47±55.15	28.99±1.49	32.72±1.90	81.99±6.81
	eight weeks later	43.88±1.83	14.82±0.67	132.23±37.19	29.25±1.77	32.86±1.63	82.66±4.90
	P-value	0.155	0.258	0.908	0.531	0.770	0.603

* Significant with more than 95% confidence (P-value <0.05)

Table V. Comparison of mean blood indices in all three groups (comparison between groups) at the beginning of the study and eight weeks later

Blood parameter / Group		Hematocrit (%)	Hemoglobin (g/dl)	Ferritin (ng/ml)	MCH (pg)	MCHC (g/dl)	MCV (fl)
	Healthy (A)	44.03±1.48	15.08±1.06	134.53±55.83	29.82±1.41	32.67±1.99	83.18±3.45
	Control (C)	43.49±1.27	14.66±0.73	133.47±55.15	28.99±1.49	32.72±1.90	81.99±6.81
	P-value	0.213	0.137	0.952	0.101	0.935	0.445
	Healthy (A)	44.03±1.48	15.08±1.06	134.53±55.83	29.82±1.41	32.67±1.99	83.18±3.45
beginning of the study	Case (B)	43.13±1.53	14.84±0.58	135.88±59.51	29.36±1.27	32.92±2.02	83.62±3.26
	P-value	0.137	0.411	0.946	0.309	0.595	0.734
	Case (B)	43.13±1.53	14.84±0.58	135.88±59.51	29.36±1.27	32.92±2.02	83.62±3.26
	Control (C)	43.49±1.27	14.66±0.73	133.47±55.15	28.99±1.49	32.72±1.90	81.99±6.81
	p-value	0.486	0.466	0.906	0.482	0.767	0.367
	Healthy (A)	43.94±1.92	15.00±0.83	147.03±71.85	29.28±1.30	33.00±1.86	83.63±2.87
	Control (C)	43.88±1.83	14.82±0.67	132.23±37.19	29.25±1.77	32.86±1.63	82.66±4.90
	P-value	0.930	0.427	0.442	0.941	0.842	0.403
eight weeks later	Healthy (A)	43.94±1.92	15.00±0.83	147.03±71.85	29.28±1.30	33.00±1.86	83.63±2.87
	Case (B)	45.85±1.67	15.87±0.57	124.68±37.25	30.91±1.59	34.16±2.11	85.91±3.09
	P-value	0.004 *	<0.001 *	0.280	0.013 *	0.016 *	0.029 *
	Case (B)	45.85±1.67	15.87±0.57	124.68±37.25	30.91±1.59	34.16±2.11	85.91±3.09
	Control (C)	43.88±1.83	14.82±0.67	132.23±37.19	29.25±1.77	32.86±1.63	82.66±4.90
	P- value	0.001*	0.001*	0.569	0.025*	0.021*	0.032 *

* Significant with more than 95% confidence (P-value <0.05) based on Paired T-test

Discussion

All patients participating in three groups in this study were male which was different from studies of e Kahn et al. (18) and Rai et al. (19) considering women and men (regardless of gender) but similar to the studies of Mranali et al. (20), Malhotra et al. (21), and Agarwal et al. (22) where only males were studied. The reason for

this is increased risk of anemia in women due to their specific physiological conditions such as menstruation.

Patients in both groups B and C were similar in terms of periodontal conditions having no significant difference in mean of periodontal indices (Table V). Sample size in this study (N=18 in each group) was less than the limited studies where periodontal treatment was conducted. For example, in the study of Malhotra et al (21) number of participants (N=20 in each group) was lower than in the study of Kahn et al (19) (N=11 in each group). In addition, number of participants was relatively lower in comparison with studies which only examined the periodontal condition and blood indices (1, 2, 21, 23, 34). It should be noted that recent studies (without intervention) were merely descriptive having no frequent problems related to clinical trial.

In terms of the time interval of re-evaluating blood indices in the follow-ups, intervals of eight-weeks were selected in present study. In the study of Rai (19) interval of three and ten weeks was considered and in the study of Bhavya (24) two months later was considered as a time to re-evaluate periodontal and blood parameters.

At the beginning of the study, all three groups were trained about the proper way of brushing and flossing. Perhaps one of the strengths of the present study compared to the previous studies with therapeutic interventions, was re-evaluation of outcome of the treatment in first follow-up visit (four weeks later). Various studies have recently attempted to examine the relationship between periodontitis and anemia but presented some conflicting results. In the case group receiving first phase of periodontal treatment, blood indices including hematocrit, hemoglobin, MCV, MCHC, MCH had a significant increase at the end of the study compared to the beginning, similar to the studies of Agarwal et al (22), Hutter (1), Pradeep (25) and Rai (19). However, results were inconsistent with studies conducted by Santosh (7) and Latha et al (26).

The level of ferritin in this study showed no significant difference in the case group, consistent with the study of Latha (26) and inconsistent with the study of Chakraborty (27) and Bhavya (24).

Parameters of probing depth measurements (PPD), clinical attachment loss (CAL), as well as bleeding index (BI), and index plaque (PI) were significantly lower eight weeks after treatment than the beginning of the study (P value <0.05). The reduction of above-mentioned indices in the case group was due to the effect of training, observing of oral hygiene, complete treatment of the first periodontal phase, and good compliance of patients. Meanwhile, the amount of bleeding on probing in this group can be explained by effect of periodontal treatment in reducing the wall inflammation and pocket depth. In addition, those who participate consciously in an oral health study observe oral hygiene unconsciously more than before (Hawthorne effect) (28).

The decrease of PPD, CAL, and BI indices in the case group was higher than the control group and was statistically significant. Furthermore, a slight reduction of all these indices was observed in the control group which was not statistically significant. Such a reduction was probably due to the effect of oral and dental health education and the use of dental floss. It should be noted that minor changes of BOP in control group indicated that such change was not immediate and required a real reduction in inflammation of periodontal pocket wall. In all three groups, all the studied blood indices were within normal range at the beginning of the study while there was a relative reduction among the selected patients. The level of blood indices including hematocrit, hemoglobin, MCH, MCHC and MCV at the end of the study were increased significantly than the beginning of the study (eight weeks later) (P value <0.05). In addition, an intra-group comparison in case group showed a significant increase compared to healthy and control groups. Such an increase in concentration of hemoglobin, hematocrit, MCV, MCHC, MCH after scaling showed that patients with chronic periodontitis have a tendency to anemia (29).

The increase in blood indices related to red blood cells in present study was statistically significant in group B (an increase of 1.03 g / dL in hemoglobin in case group) but not as much as in anemia due to other inflammatory conditions such as rheumatoid arthritis (30), multiple myeloma (30), hemoglobin levels less than 10 g / dl, neoplastic conditions and fungal and parasitic infections (31) observed in some studies and may be due to the reason that other diseases have more severe inflammatory conditions than Periodontitis. Therefore, anemia due to chronic periodontitis is relatively mild. The low level of MCH, MCHC and MCV in case group compared with the increase in hemoglobin and hematocrit levels indicated that anemia associated with chronic periodontitis is of normocytic normochromic (26).

In comparing blood variables at the end of the study in case and control groups, mean hematocrit, hemoglobin, MCV, MCHC, MCH showed a significant increase in case group compared to control group at the end of the study. A significant increase in level of those parameters in case group at the end of the study indicates that periodontal treatment could play an adjunctive role in improvement of blood indices.

In the present study, no relationship was found between level of Ferritin and periodontal treatment. The question is whether increase in Ferritin serum levels is associated with inflammation or chronic inflammation considering its wide normal range (30-230). However, there is a lack of resources and information to establish a correlation between serum Ferritin level and periodontal disease. Therefore, further research is required for validating this relationship. The results of this study are inconsistent with a study by Prakash in 2012 indicating that serum Ferritin levels are lower in individuals with periodontitis (32).

The results of this study suggest risk of ACD due to chronic periodontitis. However, whether level of anemia is different in various degrees of inflammation and whether reducing inflammation can help to treat a moderate or severe anemia caused by chronic illness is unknown (33).

Khan et al. (18) stated the patients with severe periodontitis showed better recovery in blood indices after treatment. The outcome of this study is in contrary to the study of Khan. As the number of patients with severe periodontitis in our study was not enough (4 out of 18) in case group, comparison between moderate and severe patients could not be performed. Further studies are required for investigating the relationship between the severity of periodontal disease and its effects on systemic health of individuals.

Limitations of the study

Patient's compliance to attend follow-up visits was really challenging, so we had a steady decline in number of samples which should be replaced by new ones. Some patients were reluctant to give two blood samples in 8-weeks interval. Selection of patients with certain anemia level and then subsequent delay in their treatment for 8 weeks, was not possible because of ethical considerations so we had to choose patients with normal blood parameters whose indices should have been increased according to the opinion of our hematologist.

Conclusion

A significant increase was seen between the levels of hematocrit, hemoglobin, MCH, MCHC and MCV in group B after periodontal treatment and it was concluded that chronic periodontitis may lead to a decrease in blood indices as normocytic normochromic.

Clinical significance

The relevance and effect of periodontal treatment on ACD was mentioned in some studies. Therefore, diagnosing and modifying a chronic inflammatory process, such as periodontitis, can help to improve anemia condition.

Conflict of interest

There is no conflict of interest.

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