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Evaluation of Serum Ferritin, Hemoglobin, Mean Cell Volume, Mean Corpuscular Hemoglobin Concentration and Mean Corpuscular Hemoglobin Levels in Blood from Patients with Different Severities of Periodontal Diseases.

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ABSTRACT

Gingivitis is the inflammation of the soft tissue surrounding the teeth without attachment loss, periodontitis is the inflammation of the supporting tissues of the teeth that result in loss of connective tissue attachment and supporting bone. Anemia of chronic disease (ACD) is cytokine mediated anemia that occurs due to underlying chronic inflammatory condition or malignancies that is not due to bone marrow deficiency. Both ACD and periodontal disease caused by immune activation of the same cytokines. Determine and compare the clinical periodontal parameters (plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL)) and serum ferritin, Hemoglobin (Hb), Mean cell volume (MCV), Mean corpuscular hemoglobin Concentration (MCHC) and Mean corpuscular hemoglobin (MCH) levels in blood and assess the correlations between them at patients had gingivitis, chronic periodontitis (CP) with different severities (mild, moderate and severe) with healthy periodontium subjects. 150 male subjects with an age range between (35-50) years old were included in this study. They were divided into three groups: group of 30 patients had gingivitis, group of 90 patients had CP which further classified into (Mild CP=30 patients, Moderate CP =30 patients, Severe CP =30 patients) and 30 subjects had clinically healthy periodontium as control group. After collection of blood samples, the levels of Hb, MCV, MCH, MCHC were evaluated by automated blood analyzer and the biochemical analysis of serum ferritin was done by Enzyme linked Immune Sorbent Assay (ELISA) test. The correlations between the clinical periodontal parameters with blood parameters were almost non significant but, with Hb was mostly significant negative correlations. Comparisons among groups and subgroups revealed highly significant difference in MCHC and non significant differences in Hb, MCH, MCV and serum ferritin. Mean values of Hb, serum ferritin and MCV increased with the increase in the severity of periodontal diseases while, MCHC and MCH decreased. Periodontal diseases, through inflammatory and immune responses, influenced various hematologic parameters which could lead to anemia of chronic disease.

Keywords: Anemia of chronic disease, periodontitis, gingivitis, ferritin.

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INTRODUCTION

Anemia of chronic disease (ACD) is anemia that happens in chronic infection, inflammatory or neoplastic conditions in which there is no deficiency in bone marrow with sufficient iron and vitamins storage. In ACD the production of cytokines like: tumor necrosis factor (TNF-α), interleukin-1(IL-1) and the interferon are increased, which in turn activate inflammatory condition or immune response [1]. The accumulation of plaque on the tooth surface and along the gingival margin initiates an inflammatory reaction in the host tissue, that 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 which is known as periodontitis. This destruction is caused either by direct toxic effect of a group of gram negative bacteria 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 and a combination of both. The exaggerated host response results in elevated levels of pro-inflammatory cytokines also the release of high amounts of acute phase reactants like C-reactive protein. This lead to conditions that can be addressed as systemic inflammation. The increased levels of proinflammatory cytokines alters the iron homeostasis, erythroid progenitor proliferation, production of erythropoietin and the life span of red blood cells all of which contribute to development of ACD [2].Screening tests are performed to determine whether anemia is present and to evaluate the production and destruction of erythrocyte [3]. Ferritin is the main protein for the storage of iron inside the cell [4, 5]. Serum ferritin is the most suitable laboratory test to evaluate iron stores since it correlates with the total iron store in the body under steady state conditions [6]. The purpose of this study was to evaluate the levels of systemic hematological markers to find out whether periodontal diseases with different severities are contributors to ACD.

MATERIALS AND METHODS

This study included 150 males with age range of (35-50), years they were recruited from Periodontics Department, at the teaching hospital, in the College of Dentistry, University of Baghdad as well as from blood bank in Baghdad. The inclusion criteria were: apparently systemically healthy subjects, at least twenty teeth present, all teeth included except third molar and patients with chronic periodontitis must have a minimum of four sites with probing pocket depth of ≥ 4 mm or more and clinical attachment loss of (1-2) mm or more, this was carried out according to the international classification system for periodontal disease [7]. Patients with gingivitis presented the signs and symptoms of gingival inflammation [8] and with no periodontal pocket or clinical attachment loss While, subjects with clinically healthy periodontium had no signs and symptoms of gingival inflammation and no periodontal pocket or clinical attachment loss. The exclusion criteria were: females, smokers, alcohol drinkers, patients undergone periodontal treatment and/or used a course of anti-inflammatory, antimicrobial or other medications (ex: iron supplement) in the 3 months before the study and presence of systemic diseases. The subjects were divided into three groups: group of 30 patients had gingivitis, group of 90 patients had CP that subdivided into (mild CP=30, moderate CP= 30, severe CP= 30) according to the severity of clinical attachment loss (9) and control group 30 subjects with clinically healthy periodontium. Full medical, dental history and informed consent were taken from all participants. Clinical periodontal parameters examination was done using Marquis periodontal probe which included: (PLI (10), GI (8), BOP and PPD (11) and CAL (9)). Four sites were examined for each tooth (mesial, buccal/ labial, distal and lingual/ palatal). Under a strict aseptic condition about 5 ml venous blood were collected from each subject from ante - cubital fossa by venipuncture using 20-gauge needle with 5 ml syringe. Each blood sample was divided and collected in 2 tubes; 2.5 ml put in Ethylene diamine tetra acetic acid tube then blood analysis were done for Hb, MCV, MCH and MCHC evaluation by automated blood analyzer, and 2.5 ml put in gel plane tube left in stand position and allowed to clot at room temperature for 30 minutes before centrifugation for 15 minutes at 1000 rpm to separate serum from blood, then serum collected in plane tubes which kept in the deep freeze at -80 ºC till used for subsequent biochemical analysis of serum ferritin using ELISA test.In this study the statistics used were: Standard deviation (S.D.), mean, analysis of variance test, Least significant difference (LSD) test and pearson's correlation coefficient (r) test. Levels of significance (Sig.) used were: Non significant (NS): $P > 0.05$, Significant (S): $0.05 \geq P > 0.01$ and Highly significant (HS): $P \leq 0.01$.We clarify that this study involving human subjects is in accordance with Helsinki declaration of 1975 as revised in 2000 and that it has been approved by the relevant institutional Ethical Committee (12).

RESULTS

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Statistically highly significant differences among groups and subgroups were found in PLI, GI, BOP Score 1, PPD and CAL, as shown in Table (1). Statistically highly significant difference among groups and subgroups was found in MCHC, while Hb, MCV, MCH and serum ferritin were non significant. The highest mean value of Hb (15.68) was at the gingivitis group and the mild CP subgroup demonstrated the lowest mean value (15.29). The highest mean value of ferritin (53.02) was at the moderate CP subgroup and the control group demonstrated the lowest mean value (42.91). While the MCV highest mean value was (87.84) at both severe and mild CP subgroups and the control group demonstrated the lowest mean value (85.78). The highest mean value of MCH (28.76) was at the control group and the mild CP demonstrated the lowest mean value was (27.76) and the highest mean value of MCHC (33.55) was at the control group and mild CP demonstrated the lowest mean value was (31.91), as shown in Table (2). The statistical analysis using LSD test for the MCHC mean values between each pair of the groups and subgroups, revealed non significant differences between gingivitis group with all CP subgroups, as well as between CP subgroups with each other. However, highly significant differences were found between control group with gingivitis group, mild and moderate CP subgroups. Also, significant difference between control group with severe CP subgroup, that can be noticed in Table (3).The results of Hb reveled highly significant strong negative correlations for PLI at moderate and severe CP hence, they were moderate negative for GI which was highly significant at severe CP but, they were significant at mild, moderate CP and gingivitis group while, highly significant strong negative for BOP score 1 at CP subgroups, as shown in Table (4). While, serum ferritin results were moderate negative correlations at mild CP, which was significant for GI and highly significant for BOP score 1, Table (5). From Table (6), the MCV results showed significant moderate negative correlations with PLI at moderate and severe CP subgroups. The results of MCH presented correlations at moderate and severe CP for PLI were significant moderate negative while, at mild CP highly significant moderate negative for BOP score 1, significant correlations at severe CP which was strong negative for PPD and moderate positive for CAL, as shown in Table (7). Significant strong negative correlation between MCHC with PPD at severe CP group, that can be noticed in Table (8).

Table1: Statistical analysis of clinical periodontal parameters for CP subgroups, Gingivitis and Control groups

Groups and subgroups	PL I		GI		BOP Score 1		PPD		CAL	
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.
Control	0.21	0.09	1.106	0.073	-		-		-	
Gingivitis	0.51	0.57	1.116	0.179	8.90	3.30	-		-	
Mild CP	1.87	0.49	1.370	0.364	22.9	13.76	4.93	0.99	1.68	0.204
Moderate CP	1.95	0.40	1.625	0.429	34.1	10.86	5.33	1.31	3.57	0.291
Severe CP	2.10	0.24	1.872	0.383	49.1	10.42	5.66	1.24	6.36	0.641
F-test without Control group	111.761		484.212		83.782		24.88		928.703	
P-value	0.000		0.000		0.000		0.000		0.000	
Sig.	HS		HS		HS		HS		HS	

Table 2: Statistical analysis of serum ferritin (µg/L), Hb (g/dL), MCV (fL), MCH (pg) and MCHC(g/dL) levels in blood for CP subgroups, Gingivitis and Control groups.

Groups and subgroups	Hb		Serum Ferritin		MCV		MCH		MCHC	
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.
Control	15.30	1.18	42.91	19.39	85.78	5.34	28.76	2.18	33.55	1.86
Gingivitis	15.68	1.59	45.23	22.50	86.61	5.95	27.99	2.25	32.13	1.33
Mild CP	15.29	1.14	45.35	22.69	87.84	4.25	27.76	2.42	31.91	1.22
Moderate CP	15.48	1.06	53.02	25.58	87.75	5.33	28.02	1.93	31.96	1.57
Severe CP	15.46	0.95	50.28	26.40	87.84	6.11	28.05	2.49	32.26	1.82
F-test	0.467		0.953		0.887		0.885		5.872	
P-value	0.760		0.435		0.473		0.475		0.000	
Sig.	NS		NS		NS		NS		HS	

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Table 3: Comparisons of mean values of MCHC parameter between all pairs of groups and subgroups.

Groups and subgroups		Mean Difference	P-value	Sig.
Control	Gingivitis	1.43	0.000	HS
	Mild CP	1.65	0.000	HS
	Moderate CP	1.61	0.000	HS
	Severe CP	1.31	0.001	S
Gingivitis	Mild CP	0.22	0.575	NS
	Moderate CP	0.17	0.657	NS
	Severe CP	-0.12	0.765	NS
Mild CP	Moderate CP	-0.04	0.909	NS
	Severe CP	-0.34	0.394	NS
Moderate CP	Severe CP	-0.30	0.461	NS

Table 4: Correlation between hemoglobin with the clinical periodontal parameters of the Gingivitis group and CP subgroups

Groups and subgroups	PLI		GI		BOP Score 1		PPD		CAL	
	r	p	r	p	r	p	r	p	r	p
Gingivitis	-0.009	0.962	-0.350	0.053	-0.388	0.063	-	-	-	-
Mild CP	-0.313	0.092	-0.409	0.025	-0.681	0.000	0.160	0.762	-0.008	0.969
Moderate CP	-0.748	0.00	-0.368	0.045	-0.767	0.000	-0.196	0.709	0.183	0.332
Severe CP	-0.985	0.000	-0.471	0.009	-0.824	0.000	-0.038	0.930	-0.009	0.961

Table 5: Correlation between serum ferritin with the clinical periodontal parameters of the Gingivitis group and CP subgroups

Groups and subgroups	PLI		GI		BOP Score 1		PPD		CAL	
	r	p	r	p	r	p	r	p	r	p
Gingivitis	0.039	0.836	-0.191	0.302	-0.036	0.849	-	-	-	-
Mild CP	0.020	0.915	-0.374	0.042	-0.574	0.001	0.077	0.885	-0.117	0.538
Moderate CP	0.219	0.245	-0.015	0.938	0.043	0.822	0.113	0.832	0.234	0.212
Severe CP	0.095	0.616	0.018	0.924	-0.068	0.721	-0.445	0.269	0.119	0.531

Table 6: Correlation between Mean Cell Volume with the clinical periodontal parameters of the Gingivitis group and CP subgroups

Groups and subgroups	PLI		GI		BOP Score 1		PPD		CAL	
	r	p	r	p	R	p	r	p	r	p
Gingivitis	-0.020	0.915	-0.109	0.558	-0.016	0.930	-	-	-	-
Mild CP	0.004	0.981	0.188	0.320	-0.161	0.397	0.481	0.334	0.222	0.238
Moderate CP	-0.354	0.055	-0.059	0.756	-0.293	0.116	0.640	0.171	-0.011	0.956
Severe CP	-0.374	0.042	-0.029	0.879	-0.183	0.333	-0.315	0.447	0.067	0.725

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Table 7: Correlation between Mean Corpuscular Hemoglobin with the clinical periodontal parameters of the Gingivitis group and CP subgroups

Groups and subgroups	PLI		GI		BOP Score 1		PPD		CAL	
	r	p	r	p	r	p	r	p	r	p
Gingivitis	-0.131	0.483	-0.186	0.316	-0.028	0.882	-	-	-	-
Mild CP	-0.080	0.674	0.001	0.998	-0.494	0.006	0.688	0.131	0.075	0.694
Moderate CP	-0.359	0.051	-0.070	0.712	-0.294	0.114	0.568	0.240	0.099	0.602
Severe CP	-0.431	0.017	0.054	0.778	-0.197	0.297	-0.709	0.049	0.424	0.020

Table 8: Correlation between Mean Corpuscular Hemoglobin Concentration with the clinical periodontal parameters of the Gingivitis group and CP subgroups

Groups and subgroups	PLI		GI		BOP Score 1		PPD		CAL	
	r	p	r	p	r	p	R	p	r	p
Gingivitis	-0.148	0.428	-0.175	0.348	-0.111	0.553	-	-	-	-
Mild CP	-0.130	0.493	-0.063	0.742	0.287	0.124	0.617	0.192	0.005	0.981
Moderate CP	-0.067	0.727	-0.037	0.845	-0.071	0.708	0.208	0.692	0.164	0.387
Severe CP	-0.139	0.465	0.062	0.744	-0.074	0.696	-0.780	0.023	0.339	0.067

DISCUSSION

The clinical periodontal parameters (PLI, GI, BOP score 1, PPD and CAL) demonstrated highly significant differences among groups and CP subgroups, this agree with other studies [13, 14]. Periodontal disease result from neglected oral hygiene and accumulated dental plaque, it is the net result of interaction between host immune-inflammatory reaction and dental plaque bacteria that lead to destruction of periodontal ligament fibers, resulting in clinical loss of attachment and resorption of the alveolar bone [11]. The results demonstrated non significant difference for Hb among groups and CP subgroups.Although the Hb levels were within normalrange, there were mild increase in the mean values and gingivitis group showed the highest value followed by moderate CP subgroup. Iron concentration controls Hb synthesis [3]. In hypothesis, ACD change iron distribution due to hepcidin hormone which produced by liver in response to the inflammatory cytokines like IL-6 which elevated during periodontal disease. The results agree with other studies [15, 16, 18, 19]. Disagree with previous studies[1, 14, 20, 21, 22, 23, 24, 25, 26, 27, 28].

The ferritin results revealed non significant difference among groups and CP subgroups**.** Ferritin plays crucial role in the host immune response and important task in the body as a protective function during infection in which the stimulated immune response causes the intracellular ferritin migration from the plasma as it plays important function in iron storage so, no iron is available to the infective agents as they try to bind iron from the host tissue. In addition to, its function as acute-phase reactant during inflammation, autoimmune disorders and liver disease and this what happens in ACD patient in which the ferritin level is normal or increased [18].In the present study, the mean levels of serum ferritin were elevated and the most elevation were predicted in CP subgroups, the highest level was evident in the moderate CP subgroup and all within normal range. This could be attributed to the fact that periodontitis is inflammatory disease and the main ACD pathogenesis is the presence of inflammation or infection [18].The results coincide with other studies [14, 17].But, disagree with previous studies [18, 29].The MCV results revealed non significant difference among groups and CP subgroups. The MCV values were in the normal reference range although there were increase in the mean values and the highest values seen in the severe as well as in the mild CP subgroups. When the MCV decreased indicates microcytosis and it's mostly resulted from iron deficiency

anemia while, elevation of the MCV indicates macrocytosis which is caused by vitamin deficiency [25]. So, the patients may have developed mild macrocytosis due to the reduction in vitamin B12 or folic acid level [23].The results agree with previous studies [14, 17, 18, 22, 25, 28], disagree with other studies [20, 21, 23, 27].The results of MCH showed non significant difference among groups and CP subgroups. The MCH values were within the normal reference range however, the mean values decreased hence, the mild CP subgroup and gingivitis group showed the lowest mean values respectively. Depressed MCH value reveal microcytosis which related to iron difficiency and its elevation indicate macrocytosis (vitamin deficiency) [22]. So, the reduction seen could be due to mild microcytosis caused by iron deficiency as there were no previous reference to the iron level and the diet [23]. These results agree with other studies [14, 17, 18, 22, 25, 28, 30] disagree with previous studies [20, 23, 27].The results of MCHC demonstrated highly significant difference among groups and CP subgroups. Although the mean values were within normal range there were gradual decrease and the lowest values seen in mild and moderate CP subgroups respectively indicating mild hypochromic anemia which has been reported in 30-40% in cases of ACD[21]. The reduction in MCHC in chronic disease could be due to decreased RBCs saturation by Hb secondary to decreased iron hemostasis in the presence of chronic inflammation which resulted from increase in the inflammatory cytokines levels such as (TNF- α, IL- 1 and IL- 6) that encountered in periodontal diseases [2]. The results coincide with other studies [18, 20, 21, 27]. But, disagree with previous studies [14, 17, 22, 23, 25, 28].The results concerning the Hb, demonstrated significant negative correlation with GI at gingivitis group, significant negative correlations with (GI, BOP score 1) at mild CP subgroup as well as with (PLI , GI ,BOP score 1) at moderate and severe CP subgroups these results in agreement with Nair et al.,[30) who revealed significant negative correlation between GI with Hb percentage at gingivitis group however, Jenabianet al.,[23] detected significant negative correlations at CP group between GI, BOP and CAL with Hb and Khan et al.,[27] showed significant negative correlations at CP groups between Hb with the clinical periodontal parameters (GI, CAL and PPD). At this study non significant positive correlation of PPD at mild CP was detected and this result agree with Aljohani[16] who revealed non significant positive correlation of PLI, BOP, PPD and CAL with Hbin her study that included periodontitis patients in different severities. The proinflammatory cytokines are stimulated by the bacteria and its products which cause suppression of bone marrow, depression of erythropoiesis and contribute to anemia [28].Significant moderate negative correlations between serum ferritin with GI and BOP score 1 at mild CP subgroup were found. Ferritin is acute phase reactant which elevated in chronic infection, inflammation and neoplastic conditions. Hence, preriodontitis is inflammatory disease and the main characteristic in ACD is presence of inflammation[18]. Unfortunately, there were lack of literature that correlate serum ferritin with clinical periodontal parameters.The MCV showed moderate correlations with PLI and PPD at CP subgroups, hence, Jenabianet al.,[23] revealed significant negative correlations at CP group between GI, BOP and CAL with MCV as well as, Khan et al.,[27]demonstrated significant negative correlations at CP groups between MCV with the clinical periodontal parameters studied (GI, CAL and PPD). The results in this study about MCHC showed almost non significant correlations with clinical periodontal parameters however, Jenabianet al.,[23] found non significant correlations between MCHC with any of hematological and clinical periodontal indices (GI, BOP score 1, PPD and CAL)) at the CP group. In addition, Nair et al.,[30] revealed non significant positive correlations for GI and negative for CAL with MCHC at gingivitis group and periodontitis group respectively. Significant negative correlation with PPD at severe CP was showed in this study hence, Khan et al.,[27] detected significant negative correlations at CP groups between MCHC with the clinical periodontal parameters studied (GI, CAL and PPD).The correlation regarding MCH were significant negative with PLI and PPD and significant positive with CAL at severe CP group, significant negative for PLI at moderate CP and highly significant negative for BOP score 1 at Mild CP however, Jenabianet al.,[23] revealed significant negative correlations at CP group between GI, BOP and CAL with MCH and Khan et al.,[27] demonstrated significant negative correlations at CP group between MCH with the clinical periodontal parameters studied (GI, CAL and PPD). In most cases of ACD the MCV, MCH and MCHC be within normal range and it mean that the anemia is normocytic, normochromic or slight hypochromic and these are the features of ACD. The MCHC is indicator of iron store in the body and in ACD as a result of decreased iron hemostasis there is decrease in the saturation of RBCs by Hb resulting from inflammatory condition such as the periodontal inflammation [2].

CONCLUSION

This study affords proofs that periodontal disease, like other chronic conditions, may lead to anemia of chronic disease.

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