Evaluation of Hematocrit Level, Red Blood Cells and White Blood Cells Counts in Blood from Patients with Different Severities of Periodontal Diseases

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ABSTRACT

Background: Anemia of chronic disease (ACD) occurs in the presence of chronic infection, inflammatory conditions or neoplastic conditions despite of adequate iron and vitamins storage. Gingivitis is the inflammation of the gingiva, periodontitis is the inflammation in the periodontium that extend deeper with loss of connective tissue attachment and supporting bone. The main pathogenesis of periodontal diseases and ACD is immune activation.

Aims of study: 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)). Evaluate the hematocrit (Hct) level, red blood cells (RBCs) count and white blood cells (WBCs) count. Assess the correlations between the clinical periodontal parameters and hematological parameters at patients had gingivitis, chronic periodontitis (CP) with different severities (mild, moderate and severe) with healthy periodontium subjects.

Materials and Methods: 35-50 years old, 150 male subjects were included in this study. They were divided into three study groups: group of 30 patients with gingivitis, group of 90 patients with CP which subdivided into (Mild CP=30 patients, Moderate CP =30 patients, Severe CP =30 patients) and control group 30 subjects with clinically healthy periodontium. Blood samples were collected then by automated blood analyzer the Hct, RBCs and WBCs were evaluated.

Results: Comparisons among groups and subgroups revealed significant differences in Hct and WBCs, while RBCs was non-significant. Means values of RBCs count showed reduction in mild and severe CP subgroups. while, the Hct and WBCs mean values increased in patients with periodontal disease. The correlations between the clinical periodontal parameters with WBCs and RBCs were almost non-significant but, with Hct was mostly significant negative correlations.

Conclusion: Inflammatory and immune responses in periodontal diseases caused change in different hematologic parameters which could contribute to the development of anemia of chronic disease.

Key words: Anemia of chronic disease, periodontitis, RBCs, WBCs, Hct. **(J Bagh Coll Dentistry 2018; 30(3): 1-6)**

INTRODUCTION

Anemia of Chronic Disease (ACD) anemia that is occurs in inflammatory conditions, infections and tumors. This type of anemia occurs despite adequate iron stores and no bone marrow dysfunction [1]. The pathogenesis of ACD is complex and multifactorial, linked to the underlying chronic disease, but mainly due to alterations in iron balance that derived from the immune activation. At least three major immune-driven mechanisms contribute to the development of ACD which are: the reduction in the life span of erythrocytes, the impaired proliferation of erythroid progenitor cells and the increased uptake and retention of iron within cells of the reticuloendothelial system [2].

The inflammation develops in the oral host tissue as long as there is plaque accumulation along the gingival margin that lead to either gingivitis in which it remains localized coronal to the junctional epithelium or to periodontitis in which it extends deeper, leading to loss of connective tissue attachment and supporting bone [3].

CP is the most familiar type of periodontal disease and mostly seen in adults which is proceeds relatively slow. [4]

There is a possible pathogenic relation between anemia and periodontal disease. Bacteria that cause periodontal diseases stimulate fibroblasts, keratinocytes and macrophages of periodontium, that leads to release of inflammatory cytokines such as tumor necrosis factor α (TNF-α), prostaglandin E_2 (PGE_2) and interleukins (IL): IL-1, IL-6 and IL-12. Cytokines can cause biological effects or tissue damages such as destruction of connective tissues and alveolar bones. The increase of some inflammatory response cytokines such as TNF-α, interferon (INF) and IL-1 have been observed in patients with ACD. Because of a similar role of cytokines in pathogenesis of both ACD and periodontitis [1]. A relationship between these two diseases was proposed. The aim of this study was to evaluate whether periodontal diseases in different severities contribute to the development of ACD by estimating Hct, the counts of red blood cells (RBCs) and white blood cells (WBCs).

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MATERIALS AND METHODS

The human sample consisted of 150 males with age range of 35-50 years. The subjects were recruited from Periodontics Department, at the teaching hospital, College of Dentistry, University of Baghdad as well as from the Iraqi national blood bank in Baghdad. Written consents were obtained from all of them. The participants divided into three groups: control group (clinically healthy periodontium), gingivitis group and CP group which subdivided according to the severity of clinical attachment loss [5] into subgroups (mild, moderate and severe CP), 30 subjects in each group and subgroup.

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 PPD of ≥ 4 mm and CAL of 1-2 mm or more. It was carried out according to the international classification system for periodontal disease [6]. Patients with gingivitis characterized by the presence of signs and symptoms of gingival inflammation [7] and without periodontal pocket or clinical attachment loss. Clinically healthy periodontium, characterized by the absence of any signs and symptoms of gingival inflammation and without periodontal pocket or clinical attachment loss. While, the exclusion criteria were: females (to eliminate bias as females are more prone to hormonal imbalance during puberty, reproductive and menopausal age [4]) 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. Full medical and dental history, medications, smoking status were taken from all participants.

Complete clinical periodontal parameters examination using Marquis periodontal probe which included: PLI [8], GI [7], BOP% and PPD [9] and CAL [5]. Four sites were examined per tooth (mesial, buccal/ labial, distal and lingual/ palatal). Then 2.5 ml of blood was collected from each subject into Ethylene diamine tetra acetic acid (EDTA) tube for Hct, RBCs and WBCs evaluation by automated blood analyzer as in sufficient blood EDTA ratio can cause clotting or hemolysis of blood sample [10].

In this study the statistics used were: Standard deviation (S.D.), mean, analysis of variance test, Bonferroni and Pearson's correlation coefficient (r) test. levels of significance (Sig.) were used: Non significant (NS): $p > 0.05$, Significant (S): $p \leq 0.05$. We clarify that this study involving human subjects is in accordance with Helsinki declaration of 1975 [11].

RESULTS

Statistically S differences among gingivitis group and CP subgroups were found in PLI, GI, BOP Score 1, PPD and CAL, as shown in table 1.

Statistically S differences among control, gingivitis groups and CP subgroups were detected in Hct and WBCs, while RBCs was NS. The highest mean value of Hct (48.79±4.05) was at the gingivitis group and the control group demonstrated the lowest mean value (45.76±4.18). The highest mean value of RBCs (5.62±0.620) was at the gingivitis group and the mild CP subgroup demonstrated the lowest mean value (5.41±0.427). The highest mean value of WBCs (7.95±2.64) was at the mild CP subgroup and the control group demonstrated the lowest mean value (6.58±1.59), as shown in Table 2.

The statistical analysis using Bonferroni test for the WBCs mean values revealed NS differences except the S difference between control group with severe CP subgroup, this can be noticed in Table 3. While, Hct mean values revealed NS differences except, the S difference between control group with gingivitis group (Table 4).

The Hct correlations results were: S negative correlations (R=0.579, R=0.690) with PLI at moderate and severe CP subgroups, for GI was S negative (R=0.435) at severe CP, for BOP score 1 they were S negative (R=0.356, R=0.551, R=0.609, R=0.614) at gingivitis group and CP subgroups, (Table 5).

The RBCs showed S negative correlations with PLI, PPD at mild CP subgroup (R=0.465, R=0.846), S negative correlation for BOP score 1 at moderate CP (R=0.417) and S negative correlation with CAL at severe CP(R=0.458) (Table 6).

The results of WBCs, revealed S positive correlation with BOP score 1 and CAL at moderate CP subgroup (R=0.453, R=0.375), as shown in Table 7.

Table 1: Statistical analysis of clinical periodontal parameters for CP subgroups, Gingivitis and Control groups (one-way ANOVA test used)

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	111.761		484.212		83.782		24.88		928.703	
p-value	0.000		0.000		0.000		0.000		0.000	
Sig.	S		S		S		S		S	

Significant (S): $p \leq 0.05$

Table 2: Statistical analysis of Hct level, RBCs count and WBCs count in blood for CP subgroups, Gingivitis and Control groups.

Groups and subgroups	Hct		RBCs		WBCs	
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.
Control	45.76	4.18	5.45	0.408	6.58	1.59
Gingivitis	48.79	4.05	5.62	0.620	7.28	1.53
Mild CP	47.98	3.91	5.41	0.427	7.95	2.64
Moderate CP	48.53	3.89	5.54	0.408	7.49	1.40
Severe CP	48.01	3.71	5.42	0.465	7.93	1.26
F-test	2.601		1.092		2.542	
p-value	0.038		0.363		0.042	
Sig.	S		NS		S	

Significant (S): $p \leq 0.05$, Hct level (percentage), RBCs count (number of cells $\times 10^{12}$/L), WBCs count (number of cells $\times 10^{9}$/L).

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

Groups and subgroups		Mean Difference	p-value	Sig.
Control	Gingivitis	-0.553	1.000	NS
	Mild CP	-1.226	0.077	NS
	Moderate CP	-0.771	0.912	NS
	Severe CP	-1.32	0.041	S
Gingivitis	Mild CP	-0.673	1.000	NS
	Moderate CP	-0.218	1.000	NS
	Severe CP	-0.769	0.924	NS
Mild CP	Moderate CP	0.454	1.000	NS
	Severe CP	-0.096	1.000	NS
Moderate CP	Severe CP	-0.550	1.000	NS

Bonferroni test used.

Table 4: Comparisons of mean values of Hct parameter between all pairs of groups and subgroups.

Groups and subgroups		Mean Difference	p-value	Sig.
Control	Gingivitis	-2.896	0.004	S
	Mild CP	-2.086	0.402	NS
	Moderate CP	-2.636	0.098	NS
	Severe CP	-2.113	0.377	NS
Gingivitis	Mild CP	0.810	1.000	NS
	Moderate CP	0.260	1.000	NS
	Severe CP	0.783	1.000	NS
Mild CP	Moderate CP	-0.550	1.000	NS
	Severe CP	-0.026	1.000	NS
Moderate CP	Severe CP	0.523	1.000	NS

Bonferroni test used.

Table 5: Correlation between Hct 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	0.071	0.704	-0.333	0.067	-0.356	0.050	-	-	-	-
Mild CP	-0.213	0.258	-0.345	0.062	-0.551	0.002	-0.083	0.875	-0.020	0.916
Moderate CP	-0.579	0.001	-0.289	0.121	-0.609	0.000	-0.240	0.647	0.065	0.731
Severe CP	-0.690	0.000	-0.435	0.016	-0.614	0.000	0.603	0.113	-0.254	0.176

Pearson's correlation coefficient (r) test used**.**

Table 6: Correlation between RBCs count 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	0.095	0.610	-0.184	0.322	-0.288	0.116	-	-	-	-
Mild CP	-0.465	0.010	-0.205	0.278	-0.116	0.540	-0.846	0.034	-0.035	0.853
Moderate CP	-0.315	0.090	-0.272	0.145	-0.417	0.022	-0.450	0.371	0.080	0.673
Severe CP	-0.256	0.172	-0.304	0.103	-0.291	0.118	0.253	0.545	-0.458	0.011

Pearson's correlation coefficient (r) test used**.**

Table 7: Correlation between WBCs count 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	0.066	0.722	0.274	0.136	0.024	0.897	-	-	-	-
Mild CP	0.124	0.514	0.197	0.297	0.196	0.299	-0.093	0.861	0.157	0.408
Moderate CP	-0.167	0.377	-0.120	0.527	0.453	0.012	0.331	0.521	0.375	0.041
Severe CP	-0.021	0.911	-0.063	0.742	-0.102	0.590	-0.085	0.841	-0.147	0.438

Pearson's correlation coefficient (r) test used

DISCUSSION

The clinical periodontal parameters (PLI, GI, BOP score 1, PPD and CAL) demonstrated S differences among gingivitis group and CP subgroups, this in agreement with other studies [12,13]. Periodontal disease result from neglected oral hygiene and accumulated dental plaque, it is the 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 [9]. The results of Hct revealed S difference among control, gingivitis groups and CP subgroups. The results coincide with other studies [1,4]. While, disagree with previous studies [14,15]. The Hct level depends on RBCs and mean corpuscular volume (MCV) levels, if one or both increased, the Hct level increase and the opposite is true [3]. The RBCs mean values increased in gingivitis group and moderate CP subgroup, hence, the Hct level was increased. This was clearly shown with maximum Hct value among the gingivitis group. The Hct is not an indication about presence of anemia or blood loss only, but also provide important indication about the blood capacity to transport oxygen as well as, the body hydration state. The Hct increased due to under hydration that decreases plasma volume [16]. Hct result showed almost S negative correlations with periodontal parameters. This could be attributed to the hydration state of the patients, as the under hydration state could cause reduction in the salivary flow rate [17]. Decreased salivary flow rate lead to enhance the plaque buildup and increase the incidence of dental caries and periodontal disease [9]. The salivary flow rate level decreased in gingivitis group and CP subgroups [18]. The RBCs results demonstrated NS difference among control, gingivitis groups and CP subgroups, although the means values were within the normal range but they showed reduction in mild and severe CP subgroups. These results coincide with previous studies [12,14]. While, disagree with other studies [1,4]. In ACD, increase in some inflammatory cytokines such as TNFα, interferon (INF) and IL-1 have been reported. These cytokines lead to decreased RBC life span, impaired development of erythroids, decrease erythropoietin response, interfere with the differentiation of erythroids [1]. The RBCs correlation results in agreement with Khan *et al.*, [15], Periodontal inflammation suggested to down regulate erythropoesis by proinflammatory

cytokines and reduce RBCs count [19]. The WBCs results revealed S difference among the control, gingivitis groups and CP subgroups. These results agree with previous studies [3,12], while they disagree with other finding [20]. This could be attributed to the activated response of total leukocytes since, they are the first line of body defense during infection and inflammation [21]. In periodontitis and gingivitis there are elevations in neutrophils numbers that result in increase in leukocytes numbers [22]. There were lack of literature which correlate WBCs count with the clinical periodontal parameters. With the incidence of subgingival pathogens exudation and migration from the nearby capillaries of large number of leukocytes to the site of infection occur that involved in the first line of defense against bacterial pathogens [23].

CONCLUSION

Periodontal diseases are mild inflammatory conditions, they are low grade infection that may lead to signs of anemia however, they are not very severe as observed in other systemic diseases that can cause ACD (e.g. Rheumatoid arthritis, malignancy and others).

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الخلاصة

الخلفية: فقر الدم الناتج من الأمراض المزمنة هو فقر الدم الذي يحدث في وجود عدوى مزمنة والحالات الالتهابية او حالات الاورام على الرغم من وجود مخزون حديد وفيتامين كافيان. التهاب اللثة هو التهاب في نسيج اللثة، التهاب الأنسجة الداعمة للأسنان الذي يمتد أعمق مع فقدان مرفق الأنسجة الضامة والعظام الداعمة. السبب الرئيسي لتطور امراض أنسجة ما حول الأسنان وفقر الدم الناتج من الأمراض المزمنة هو التنشيط المناعي

أهداف الدراسة: تحديد ومقارنة مؤشرات أنسجة ما حول الأسنان السريرية (مؤشر الصفيحة الجرثومية، مؤشر التهاب اللثة، مؤشر النزف عند التسبير، عمق جيوب اللثة ومستوى الأنسجة الرابطة سريريا) ونسبة اللزوجة في الدم، عدد كريات الدم الحمراء وعدد كريات الدم البيض في الدم وتحديد العلاقات بينهم في مجموعات الدراسة التي تشمل: مجموعة التهاب اللثة ومجموعة التهاب اللثة المزمن مع الشدة المختلفة التي قسمت الى: طفيف، معتدل وحاد مع مجموعة ضابطة (لثة صحية سريريا).

المواد والطرق: بين (50-35) سنة، 150 شخص من الذكور تم شملهم في هذه الدراسة. تم تقسيمهم الى ثلاثة مجاميع دراسية كالاتي: مجموعة التهاب اللثة (30) مريض ومجموعة التهاب اللثة المزمن (90) مريض (قسمت المجموعات فرعية وهي: التهاب اللثة المزمن الطفيف، التهاب اللثة المزمن المعتدل، التهاب اللثة المزمن الحاد (30) مريض في كل مجموعة فرعية) مجموعة ضابطة (لثة صحية سريريا) (30) شخص. تم سحب عينات الدم وبعد ذلك عن طريق جهاز تحليل الدم الذاتي تم تحديد نسبة اللزوجة في الدم، عدد كريات الدم الحمراء وعدد كريات الدم البيض.

النتائج: المقارنة بين المجموعات والمجموعات الفرعية أظهرت فرق معنوي لكل من: نسبة اللزوجة في الدم وعدد كريات الدم البيض. في حين عدد كريات الدم الحمر كان فرق غير معنوي. المتوسط الحسابي لعدد كريات الدم الحمراء اظهر انخفاض في مجموعة التهاب اللثة المزمن الطفيف ومجموعة التهاب اللثة المزمن الحاد ولكن ازداد في مجموعة التهاب اللثة ومجموعة التهاب اللثة المزمن المعتدل. في حين المتوسط الحسابي لنسبة اللزوجة في الدم وكريات الدم البيض ازداد في مرضى أمراض أنسجة ما حول الأسنان. العلاقة بين مؤشرات أنسجة ما حول الأسنان السريرية وكريات الدم البيض وكريات الدم الحمر كانت علاقة غير معنوية على الأغلب لكن مع نسبة اللزوجة في الدم كانت غالبا علاقة معنوية سلبية.

الاستنتاج: الأستجابة الألتهابية والمناعية لأمراض أنسجة ما حول الاسنان سببت تغيير في مختلف المؤشرات الدموية التي من الممكن أن تساهم في حدوث فقر الدم الناتج عن الأمراض المزمنة.

الكلمات الدالة: فقر الدم الناتج من الأمراض المزمنة، التهاب الأنسجة الداعمة للأسنان، عدد كريات الدم الحمراء، عدد كريات الدم البيض، نسبة اللزوجة في الدم.