

Research Article

Evaluation of leukocytes cells types counts in blood from patients with different severities of periodontal diseases

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Received date: 15-03-2022

Accepted date: 04-05-2022

Published date: 15-06-2023



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<https://doi.org/10.26477/jbcd.v35i2.3402>

Abstract: Background: Periodontal diseases are inflammatory disorders caused by the accumulation of oral biofilm and the host response to this accumulation which characterized by exaggerated leukocytes and neutrophils attraction to the sites of inflammation by chemoattractants which are a very important part of the pathogenesis of periodontal diseases. This study aimed to determine and compare the clinical periodontal parameters and the leukocyte cell types in the peripheral blood between patients with gingivitis and periodontitis with different severities compared to healthy controls. Materials and methods: This study included 150 male subjects aged between 35-50 years. They were divided into three groups: gingivitis group (n=30), periodontitis patients (n=90) which subdivided into Mild =30 patients, Moderate =30 patients, Severe =30 patients and a control group (n=30) with clinically healthy periodontium. Clinical periodontal parameters were recorded ((plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL)). Blood samples were collected then an automated blood analyzer evaluated leukocyte cell types. Results: Significant differences in The counts of neutrophils and lymphocytes exhibited significant differences among the study groups and subgroups. On contrary, differences in monocytes, eosinophils, and basophils counts were not significant. Additionally, severity of periodontitis was significantly correlated with the mean counts of the various leukocyte cell types; however, clinical periodontal characteristics did not show such correlation with these inflammatory cells. Conclusion: This study demonstrated that periodontal disease with different severities is associated with possible episodes of bacteremia that originate from periodontal lesions which mediate inflammatory conditions that in turn causing changes in the systemic markers especially leukocytes cells types.

Keywords: leukocytes, neutrophils, periodontal diseases

Introduction:

The inflammation develops in the oral tissues as long as plaque accumulates along the gingival margin. The inflammation may lead to either gingivitis, which remains localized coronally to the junctional epithelium, or to periodontitis, which extends deeper leading to loss of connective tissue attachment and the supporting bone. This devastating destruction is either due to the direct toxic effect of Gram-negative bacteria mainly *Porphyromonasgingivalis*, *Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia* or the host's response to potent periodontopathogens mediated first by the polymorphonuclear leukocytes (PMNs) and subsequently by the cells of the reticulo-endothelial system i.e., monocytes and macrophages.

Indeed, in periodontitis, excessive production of pro-inflammatory cytokines and acute-phase reactants such C-reactive protein in response to bacteria is the hallmark of the host's immune system ⁽¹⁾.

Leukocytes include five morphologically and functionally distinct types of nucleated blood cells: neutrophils, eosinophils, basophils, monocytes, and lymphocytes. The leukocytes are attracted to sites of inflammation, infection, or tissue injury by chemoattractant and leave the circulation using special adhesion molecules and ligands located on the leukocytes and endothelial cells of the vessel walls. Leukocytes are the major systemic cells of phagocytosis and the first cells of the host defense mechanism against infective agents. During periodontitis, neutrophils are initially predominant cells of the host defense mechanism and have a significant role in inflammation and pathogenesis. It is supposed that there is an increase in the count of these cells in periodontitis of various severities due to the accumulation of the oral biofilm when compared to healthy subjects, Loos *et al.* ⁽²⁾ and Loos *et al.* ⁽³⁾ showed high levels of neutrophils and lymphocytes in patients with periodontitis as compared to subjects with healthy periodontium. Periodontal inflammation can deteriorate systemic conditions through the pathology caused by leukocytes ⁽⁴⁾. This study aimed to assess the leukocyte cell type counts in the peripheral blood of patients suffering from different severities of periodontal diseases.

Materials and methods:

This case-control study involved 150 males aged between 35 to 50 years who were recruited from the patients who attended to Department of Periodontics, College of Dentistry, University of Baghdad as well as from the Blood Bank in Baghdad in the period from December 2016 to April 2017. The patients were divided into three groups: the 1st group consisted of 30 patients with generalized gingivitis and the 2nd group included 90 patients with periodontitis that subdivided according to the severity of clinical attachment loss where mild 1-2 mm, moderate 3-4 mm and severe ≥ 5 mm ⁽⁵⁾ (30 patients /group) and a control group (n=30) with clinically healthy periodontium.

Inclusion criteria:

1. Systemically healthy subjects with ≥ 20 teeth present.
2. All teeth except third molars included in this study and according to the international classification system for periodontal disease ⁽⁶⁾, the periodontitis group must have at least four sites with probing pocket depth of ≥ 4 mm and clinical attachment loss of 1-2 mm or more.
3. In gingivitis group, the patients are characterized by signs and symptoms of gingival inflammation ⁽⁷⁾, but without any true periodontal pockets or clinical attachment loss. In contrast, patients with clinically healthy periodontal tissues were characterized by the absence of any signs and symptoms of any inflammation, periodontal pockets and clinical attachment loss.

Exclusion criteria:

1. Females
2. Smokers
3. Alcohol drinkers
4. Patients underwent periodontal therapy and/or were consuming anti-inflammatory/antibacterial drugs, or any other medications in the last 3 months prior to the study.
5. Patients with history of any systemic diseases.

Full medical and dental histories were taken from all subjects and written consent was obtained. Clinical periodontal parameters were assessed using Marquis periodontal probe, which included: PLI ⁽⁸⁾, GI ⁽⁷⁾, BOP and PPD ⁽⁹⁾ and CAL ⁽⁵⁾. Four sites per tooth (mesial, buccal/ labial, distal and lingual/ palatal) were examined. After collecting 2.5 ml of blood into an Ethylene diamine tetra acetic acid tube, leukocyte cell types (neutrophils, eosinophils, basophils, monocytes, and lymphocytes) were evaluated by an automated blood analyzer. This study was following the revised Helsinki Declaration and it has been approved by the relevant institutional Ethical Committee ⁽¹⁰⁾.

Statistical analysis

The descriptive statistics used in this study to express clinical parameters and cell counts were the mean and Standard deviation (S.D.). Before conducting the inferential analyses, the distribution of data was checked by the Shapiro-Wilks test. Then multigroup comparisons were performed by analysis of variance (ANOVA) test followed by least significant difference (LSD) posthoc test. Pearson's correlation test determined the correlation between cell counts and clinical parameters. Significant differences were determined when p value was less than 0.5.

Results:

There were significant differences appeared among groups and subgroups in PLI, GI, BOP Score 1, PPD and CAL (Table 1). The Comparisons of the mean values of the clinical periodontal parameters between all pairs of the gingivitis group and periodontitis subgroups are shown in Table (2).

Data shown Table (3) indicated that neutrophils and lymphocytes displayed statistically significant variations among groups and subgroups, whereas basophils, eosinophils and monocytes did not. Mild periodontitis had the greatest mean neutrophil value, while the control group had the lowest value. The gingivitis group had the lowest mean lymphocyte value, while the mild periodontitis group had the greatest count. Additionally, severe versus moderate periodontitis groups showed the greatest mean value of monocytes as compared to other groups. For eosinophils, the control group had the lowest mean value of, whereas the mild periodontitis subgroup had the highest mean value. In contrast to the control group, which displayed the lowest mean value, severe periodontitis demonstrated the highest mean value of basophils.

The neutrophil cells count mean values were non-significant except for the significant differences between the control group with the gingivitis group, mild and severe periodontitis subgroups together with mild with moderate periodontitis subgroups. Lymphocyte cells' mean values demonstrated non-significant differences except for the significant differences between moderate periodontitis and control and gingivitis groups. Monocyte cells' mean values showed non-significant differences except the significant difference between mild with severe periodontitis subgroups (Table 4).

The correlation between leukocyte cell types and the clinical periodontal parameters was non-significant (Table 5). However, neutrophils correlations were significantly positive for GI in the gingivitis group, as well as highly significantly negative for BOP score one and positive for CAL with moderate periodontitis. In contrast, lymphocytes revealed a significant strong positive correlation with PPD in the severe periodontitis subgroup only. Monocyte count demonstrated a significant moderate negative correlation with PLI in the moderate periodontitis subgroup. Eosinophils showed non-significant correlations with all clinical periodontal parameters in all gingivitis and periodontitis subgroups. The results of basophils revealed a significant moderate positive correlation with GI in the severe periodontitis subgroup.

Table 1: 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	0.106	0.035	-		-		-	
Gingivitis	1.51	0.57	1.116	0.179	8.90	3.30	-		-	
Mild CP	1.87	0.49	1.370	0.364	22.9	10.76	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	

Table 2: Comparisons of the mean values of the clinical periodontal parameters between all pairs of CP subgroups and the Gingivitis group by using the LSD test:

Group and subgroups	PLI		GI		BOP score 1		PPD		CAL	
	Mean difference	P-value	Mean difference	P-value	Mean difference	P-value	Mean difference	P-value	Mean difference	P-value
Mild P	-0.367	0.000	-0.307	0.000	-14.00	0.000	-	-	-	-
Moderate P	-0.438	0.000	-0.550	0.000	-25.23	0.000	-	-	-	-
Severe P	-0.590	0.000	-0.778	0.000	-40.28	0.000	-	-	-	-
Mild P	-0.071	0.495	-0.242	0.000	-11.23	0.000	-0.40	0.001	-1.89	0.000
Severe P	-0.222	0.033	-0.470	0.000	-26.27	0.000	-0.73	0.000	-4.68	0.000
Moderate P	-0.151	0.146	-0.228	0.000	-15.04	0.000	-0.33	0.001	-2.79	0.000

Table 3: Statistical analysis of neutrophils, lymphocytes, monocytes, eosinophils and basophils cells count (No. of cells $\times 10^9/L$) for CP subgroups, Gingivitis and Control groups by using analysis variance test:

Groups and subgroups	Neutrophils		lymphocytes		Monocytes		Eosinophils		Basophils	
	Mean	\pm S.D.	Mean	\pm S.D.	Mean	\pm S.D.	Mean	\pm S.D.	Mean	\pm S.D.
Control	3.67	0.98	2.26	0.584	0.423	0.152	0.197	0.016	0.056	0.025
Gingivitis	4.56	1.58	2.22	0.592	0.471	0.175	0.201	0.013	0.065	0.029
Mild CP	4.91	2.12	2.36	0.531	0.406	0.165	0.210	0.013	0.067	0.025
Moderate CP	4.04	1.03	2.67	0.761	0.466	0.164	0.216	0.012	0.063	0.036
Severe CP	4.50	1.23	2.50	0.691	0.495	0.191	0.205	0.011	0.068	0.023
F-test	2.978		2.580		1.533		0.136		0.620	
P-value	0.021		0.040		0.196		0.969		0.649	

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

Groups and subgroups		Mean Difference	P-value	Sig.	
neutrophil cells	Control	Gingivitis	-0.824	0.029	S
		Mild CP	-1.170	0.002	S
		Moderate CP	-0.304	0.422	NS
		Severe CP	-0.766	0.044	S
	Gingivitis	Mild CP	-0.345	0.359	NS
		Moderate CP	0.520	0.168	NS
		Severe CP	0.058	0.877	NS
	Mild CP	Moderate CP	0.865	0.024	S
		Severe CP	0.403	0.288	NS
		Moderate CP	Severe CP	-0.462	0.224

Groups and subgroups		Mean Difference	P-value	Sig.	
lymphocyte cells	Control	Gingivitis	0.0470	0.774	NS
		Mild CP	-0.092	0.574	NS
		Moderate CP	-0.409	0.014	S
		Severe CP	-0.235	0.154	NS
	Gingivitis	Mild CP	-0.139	0.394	NS
		Moderate CP	-0.456	0.006	S
		Severe CP	-0.282	0.085	NS
	Mild CP	Moderate CP	-0.317	0.056	NS
		Severe CP	-0.143	0.386	NS
	Moderate CP	Severe CP	0.174	0.292	NS

Groups and subgroups		Mean Difference	P-value	Sig.
Monocyte cells	Gingivitis	-0.055	0.201	NS
	Mild CP	0.009	0.823	NS
	Moderate CP	-0.050	0.249	NS
	Control Severe CP	-0.079	0.070	NS
	Gingivitis Mild CP	0.065	0.136	NS
	Moderate CP	0.005	0.906	NS
	Severe CP	-0.023	0.585	NS
	Mild CP Moderate CP	-0.060	0.173	NS
	Severe CP	-0.089	0.044	S
	Moderate CP Severe CP	-0.029	0.510	NS

Table 5: Correlation between (leukocytes cells count with the clinical periodontal parameters of the Gingivitis group and CP subgroups (Person’s correlation coefficient:

Neutrophils	PLI		GI		BOP Score 1		PPD		CAL	
	r	p	r	p	r	p	r	p	r	P
Gingivitis	0.097	0.604	0.376	0.037	-0.161	0.388	-	-	-	-
Mild CP	0.038	0.841	0.155	0.412	0.151	0.427	-0.034	0.949	0.022	0.907
Moderate CP	-0.104	0.586	-0.137	0.471	-0.492	0.006	0.381	0.457	0.399	0.002
Severe CP	0.140	0.460	0.054	0.777	0.041	0.829	-0.517	0.190	-0.166	0.382
Lymphocytes	PLI		GI		BOP Score 1		PPD		CAL	
	r	p	r	p	r	p	r	p	r	P
Gingivitis	0.031	0.870	-0.295	0.107	0.197	0.287	-	-	-	-
Mild CP	0.083	0.663	0.067	0.726	-0.118	0.535	0.256	0.625	-0.178	0.348
Moderate CP	-0.231	0.218	-0.083	0.661	-0.167	0.377	0.078	0.884	0.062	0.743
Severe CP	-0.184	0.330	-0.132	0.486	-0.225	0.232	0.690	0.058	0.005	0.977
Monocytes	PLI		GI		BOP Score1		PPD		CAL	
	r	p	r	p	r	p	r	p	r	P
Gingivitis	-0.031	0.868	0.024	0.898	0.008	0.964	-	-	-	-
Mild CP	0.106	0.579	0.007	0.970	-0.003	0.986	-0.691	0.128	-0.002	0.992
Moderate CP	0.416	0.022	0.023	0.904	-0.331	0.074	0.300	0.564	0.111	0.558
Severe CP	0.130	0.494	0.140	0.460	0.056	0.768	-0.308	0.457	-0.150	0.429
Eosinophils	PLI		GI		BOP Score1		PPD		CAL	
	r	p	r	p	r	p	r	p	r	P
Gingivitis	-0.054	0.773	-0.186	0.316	0.187	0.313	-	-	-	-
Mild CP	0.195	0.301	0.124	0.513	0.103	0.587	-0.505	0.307	-0.112	0.554
Moderate CP	-0.295	0.114	0.016	0.935	-0.213	0.258	-0.086	0.872	-0.014	0.943
Severe CP	0.102	0.591	-0.039	0.839	0.067	0.725	0.001	0.998	-0.225	0.233
Basophils	PLI		GI		BOP Score1		PPD		CAL	
	r	p	r	p	r	p	r	p	r	P
Gingivitis	0.301	0.100	-0.066	0.722	0.126	0.499	-	-	-	-
Mild CP	0.002	0.992	-0.004	0.982	0.266	0.155	-0.428	0.397	-0.065	0.731
Moderate CP	-0.184	0.331	0.051	0.788	-0.102	0.590	0.033	0.951	0.117	0.539
Severe CP	0.291	0.119	0.443	0.014	0.303	0.104	-0.384	0.348	-0.292	0.117

Discussion:

According to the available literature ^(4,11), the clinical periodontal indices (PLI, GI, BOP score 1, PPD, and CAL) showed extremely significant variations between groups and periodontitis subgroups. When oral hygiene is neglected and dental plaque builds up, it causes periodontal disease. This condition is the end result of a host immune-inflammatory reaction that interacts with dental plaque bacteria to cause the destruction of periodontal ligament fibers, which causes clinical loss of attachment and resorption of the alveolar bone ⁽⁹⁾. Significant variations in the neutrophils were seen between the various groups and subgroups of periodontitis. These results coincide with previous studies ^(1-2, 12-14). Periods of bacteremia in periodontitis or lipopolysaccharide leakage to the systemic circulation result in greater amounts of neutrophil discharge since they are the first line of defense in the innate immune system ⁽¹⁵⁾. According to other investigations ^(2,3,12,14,16,17), lymphocytes demonstrated considerable variations between groups and periodontitis subgroups, but disagrees with other studies ^(4,13). When the severity of periodontal disease has been increased, there were increased numbers of neutrophils in the connective tissue and the appearance of macrophages, lymphocytes, plasma cells and mast cells. The metabolic products of bacteria trigger junctional epithelium cells to produce cytokines, causing vasodilatation of local blood vessels, and permitting the gradual migration of macrophages, plasma cells, and T and B lymphocytes from the capillaries that become the principal cells ⁽¹⁸⁾. Consequently, activated inflammatory response and the continued presence of bacterial plaque, the destruction in the periodontal ligament and bone resorption can be noticed clinically ⁽⁹⁾.

The count of monocytes, eosinophils, and basophils demonstrated non-significant differences among the groups and periodontitis subgroups. However, there was an increase in the mean cell counts with the severity of the periodontal disease. The elevated leukocyte numbers in periodontitis and experimental gingivitis have been proposed to be mainly due to the elevation in neutrophil numbers ⁽¹⁵⁾. Since the normal percentage ranges of white blood cells types are; neutrophils: 54% to 62%, lymphocytes: 25% to 33%, monocytes: 3% to 9%, eosinophils: 1% to 3% basophils: Less than 1% ⁽¹⁹⁾. Systemic infections or conditions must be severe to affect these cells but periodontal diseases are mild inflammatory conditions showed little impact on their counts ^(20,21). Therefore, periodontal inflammation could be mild that not significantly affect the counts of cells that are present in small percentages. Results of monocyte counts agree with other studies ^(12,13), but disagree with Patil ⁽¹⁾. The eosinophils results coincide with previous studies ^(12,13) while the results of basophils were consistent with other findings ^(12,13).

Local inflammatory response developed with the incidence of sub gingival pathogens which is characterized by a large number of leukocyte exudation and migration from the systemic circulation to the affected site that is involved in the first line of defense against bacterial pathogens. In addition, this inflammatory response is augmented as a result of pro-inflammatory cytokines and prostaglandins production which are formed by a variety of cells as a response to the microbial invasion such as monocytes/macrophages, neutrophils, lymphocytes, adipocytes and fibroblasts. Recruitment of more proinflammatory mediators and leukocytes to the site of infection as a result of their release in the bloodstream ⁽¹²⁾.

Basophils numbers revealed moderate correlations with GI and PPD in periodontitis patients while eosinophils demonstrated a moderate negative correlation with PPD at mild periodontitis. During infection and inflammation, there is an increase in total leukocyte count which is crucial in this context ⁽²²⁾. The periodontal lesions cause bacteremia that changes the systemic markers in the body as the disease progress and there will be an area of ulceration in the epithelial lining of the periodontal pocket that enables greater changes and stimulation of the host response to the bacterial product as manifested by an increased inflammatory response in the form of elevated total leukocyte counts ⁽⁴⁾. In general, there is a scarcity of

researches that link the circulatory inflammatory cells with periodontitis which was emphasized in the current investigation.

Conclusions:

Sequalae of Periodontal diseases with different severities are not restricted to the periodontal tissue only. Still, they also cause inflammatory states systemically influencing the counts of leukocyte cell types specially neutrophils and lymphocytes.

Conflict of interest: None.

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العنوان : تقييم أنواع خلايا كريات الدم البيضاء في دم المرضى الذين يعانون من شدة مختلفة من امراض اللثة

الباحثون: جنار جبار علي , ندى كاظم عمران, مها عبدالعزيز احمد

المستخلص:

أمراض اللثة هي اضطرابات التهابية ناتجة عن تراكم الاغشية الحيوية الفموية واستجابة العائل لهذا التراكم والتي تتميز بتضخم الكريات البيضاء والعدلات في مناطق الالتهاب بواسطة المعالج الكيميائي والتي تعد جزءاً مهماً جداً في التسبب في امراض اللثة . كان الهدف من هذه الدراسة هو تحديد ومقارنة المتغيرات السريرية اللثوية وأنواع خلايا الكريات البيضاء في الدم المحيطي بين مرضى التهاب اللثة والتهاب دواعم السن بدرجات مختلفة مقارنة بالضوابط الصحية. شملت هذه الدراسة (150) من الذكور تتراوح أعمارهم بين (35-50) سنة و تم تقسيمهم الى ثلاث مجموعات: مجموعة التهاب اللثة (ن = 30), مرضى التهاب دواعم السن (ن = 90) والتي قسمت الى معتدلة = 30 مريضاً , متوسطة = 30 مريضاً , شديدة = 30 مريضاً و مجموعة أساسية (ن = 30) . تم تسجيل معاملات اللثة السريرية ((مؤشر البلاك (PLI) ، مؤشر اللثة (GI) ، النزيف عند السبر (BOP) ، فحص عمق الجيب (PPD) ومستوى التعلق السريري (CAL)) وتم تقييمها عن طريق محلل الدم الآلي و أظهرت المقارنات بين المجموعات والمجموعات الفرعية فروق معنوية في الخلايا العدلات والخلايا الليمفاوية وفروق غير معنوية في الخلايا الأحادية ، الحمضات ، الخلايا القاعدية وأرتفعت القيم المتوسطة لأنواع خلايا الكريات البيضاء مع زيادة شدة أمراض اللثة و كان الارتباط بين المعلمات السريرية اللثوية لأنواع خلايا الكريات البيضاء غير معنوي . أظهرت هذه الدراسة أن أمراض اللثة ذات الشدة المختلفة مرتبطة بنوبة تجرثم الدم المحتملة والتي تنشأ من آفات دواعم الأسنان التي تتوسط حالة التهابية تسبب بدورها تغييرات في الواسمات الجهازية وخاصة أنواع خلايا كريات الدم البيضاء.