Dentistry 3000
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Evaluation of Salivary Interleukin 6 and MicroRNA 146a in Patients with Chronic Periodontitis and Their Association with Periodontal Parameters

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Abstract

Objective: The aim of this study was to evaluate of the role of interleukin 6 (IL-6) and microRNA 146a (miRNA 146a) in chronic periodontitis (CPD).

Patients and Methods: This cross-sectional case control study was conducted at the Diyala province-Iraq. A total of 40 CPD patients were included. They were 20 males and 20 females, and their age range was 23-56 years. The clinical periodontal parameters were measured under supervision of specialized dentists. Furthermore, 40 apparently healthy individuals, 21 were males and 19 were female, were included as controls. Their age range was 20-50 years. All participants were requested to complete a questionnaire that contained medical and socio-demographic data. The salivary IL-6 was determined using qELISA (INOVA Biotech Co., LTD, China). The salivary miRNA 146a was detected using qPCR (TransGen biotech, China). Statistical analyses were done using SPSS version 22 (Inc., Chicago, Illinois, United States), and P-values ≤ 0.05 were considered significant.

Results: The mean age ± SD of patients was not different than that of controls (38.85 ± 5.30 Vs 40.52 ± 6.69, P=0.2). The gender was also not different (P=0.8). The mean ± SD PI of CPD patients was significantly higher (1.27± 0.34 Vs 0.60 ± 0.26, P=0.01). Similarly, the mean ± SD of GI was also significantly higher in CPD patients (1.49 ± 0.42 Vs 0.24 ± 0.14, P= 0.01). The rate of BOP sites in CPD patients was significantly higher than that of controls (P=0.001). Also, the mean ± SD of PPD in CPD patients was 4.66 ± 0.50 mm, while the mean ± SD of CAL was 4.90 ± 1.30. Additionally, the mean No.± SD of teeth in CPD patients was significantly lower (24.72± 3.77 Vs 27.62± 0.80, P=0.01). Furthermore, the mean No. ± SD of sites in CPD patients was also significantly lower (98.95± 15.08 Vs 110.50 ± 3.22, P 0.01). The mean concentration ± SD of salivary IL-6 was not higher in CPD patients (8.17 ± 3.79 Vs 7.45± 1.51, P= 0.4). qPCR results found that there was significantly higher expression of miRNA-146a in CPD patients versus that of the controls (1.79 ± 0.10 Vs 1.08± 0.10, P=0.001).

Conclusion: The current results concluded that both IL-6 and miRNA 146a were elevated in CPD patients and closely correlated with periodontal parameters suggesting that these biomarkers can be used as a surrogate diagnostic and prognostic indicator of chronic periodontitis.

Keywords: chronic periodontitis; salivary IL-6; salivary microRNA146a.

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Introduction

Periodontitis (PD) is a persistent multifactorial inflammatory condition caused by the accumulation of dental plaque and eventually leads to teeth loss. Periodontitis is characterized by gingival inflammation, clinical attachment loss, pathologic migration, sites with profound probing depths, mobility, hemorrhage upon probing, and radiographic evidence of alveolar bone loss [1]. Gingival inflammation and osseous damage are mostly asymptomatic. Consequently, individuals may mistakenly interpret painless bleeding during dental cleaning as benign, despite it potentially indicating advancing periodontitis. The primary cause of gingivitis is insufficient oral hygiene, leading to the accumulation of mycotic and bacterial matrices at the gum line, referred to as dental plaque [2]. Additional factors include

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.843

inadequate nutrition and underlying conditions. medical conditions such as diabetes mellitus (DM) [3]. Genetic factors may modify the risk of developing periodontitis [4].

Numerous immune and non-immune cells, such as macrophages, dendritic cells, endothelial cells, and fibroblasts, may release interleukin 6 (IL-6), a lymphocyte chemoattractant factor [5]. Interleukin-6 (IL-6) is a crucial cytokine that plays a significant role in modulating the host's response to bacterial infections [6]. Several studies were focused on the importance of IL-6 in the development of many immune-mediated diseases, including PD [7]. Elevated IL-6 levels correlate with heightened osteoclastic activity in the alveolar bone area and an abundance of periodontal-pathogenic bacteria [8]. Moreover, IL-6 levels are used to ascertain the relationship between periodontal disease and systemic conditions, such as diabetes mellitus [9]. The local elevation of IL-6 levels in gingival crevicular fluid and saliva was linked with the occurrence of gingivitis. Moreover, IL-6 levels were markedly increased in individuals with severe or progressing forms of Parkinson's disease compared to the early stage [10].

MicroRNAs are a type of short (19–24 nucleotides) non-coding RNAs that regulate gene expression and possess important biological activities. It was found that periodontitis promotes the periodontal tissue levels of miRNAs [11], which may also change miRNA profile in saliva [12]. Alterations in the expression levels of miRNAs, either via overexpression or under expression, might result in modifications to gene expression that may influence the initiation and/or clinical advancement of periodontitis [13]. MiRNA dysregulation may be identified in several biological fluids, including serum, saliva, urine, gingival crevicular fluid, and cerebrospinal fluid. This renders them exemplary prospects as precise as well as accurate biomarkers for the diagnosis and prognosis of several illnesses [14]. MiRNA 146a, was the only reliable predictor of periodontitis among subjects with DM, i.e. MiRNA-146a Saliva provides accurate, non-invasive, diagnostic, and prognostic indicators that may be utilized to evaluate periodontal health status among DM and non-DM patients [15]. Additionally, miRNA-146a was among the most widely explored miRNA in periodontal diseases beside the miRNA-200b, miRNA-223, miRNA-23a, and miRNA-203, and all of them except miRNA-203 were found to had acceptable diagnostic plausibility for periodontitis [16].

Patients and Methods

This is a cross-sectional case control study conducted in Diyala province-Iraq for the period from October 2023 to September 2024. A total of 40 patients with chronic periodontitis (CPD) were included in this study [17]. They were 20 males and 20 females chosen from the patients treated at the center for dentistry in Baquba. The age range of these patients was 23-56 years. The clinical periodontal parameters were measured under supervision of specialized dentists. All patients were asked to fill a questionnaire including information about age, past periodontal treatment, medical history, medication used and smoking or alcohol consumption. Furthermore, 40 apparently healthy individuals, 21 were males and 19 were females, without CPD as control group. Their age range was 20-50 years. All participants gave verbal consent prior to participation in the study.

The salivary IL-6 was determined using quantitative ELISA technique

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.843

(INOVA Biotech Co., LTD, China). The salivary miRNA 146a was detected using quantitative polymerase reaction (TransGen biotech, China). The Statistical Package of Social Science (SPSS) version 22 (SPSS Inc., Chicago, Illinois, United States) was used to perform statistical analysis on the data. P values ≤ 0.05 were considered significant.

Results

Table 1 showed that Mean age ± standard deviation (SD) of patients was 38.85 ± 5.30 years while that of the control group was 40.52 ± 6.69 years (P=0.2) (Table 1).

Table 2 showed that the mean ± SD periodontal index (PI) of CPD group was significantly higher than that of controls (1.27± 0.34 Vs 0.60 ± 0.26, P=0.01). Similarly, the mean ± SD of gingival index (GI) in CPD group was significantly higher versus that of controls (1.49 ± 0.42 Vs 0.24 ± 0.14, P= 0.1).

The number and percentage distribution of different sites according to the presence or absence of bleeding on probing (BOP) are shown in Table 3. The number of sites examined for the CPD group was 3958 versus 4420 in the control group. In the CPD group, the number of BOP sites was 3127 with a rate of 79% against 831 of non-bleeding sites with a rate of 20.99%. In the control, none of the examined site showed BOP, thus all (100%) had no BOP. The difference between the two groups was statistically significant (P<0.05). Data are shown in Table 3.

The mean ± SD of periodontal pocket depth (PPD) in CPD patients was 4.66 ± 0.50 mm, while the mean ± SD of clinical attachment loss (CAL) was 4.90 ± 1.30 mm. The control individuals had no PPD and CAL. Additionally, the mean ± SD of No. of teeth in CPD patients was significantly lower than controls (24.72± 3.77 Vs 27.62± 0.80, P=0.01). Furthermore, the mean ± SD no. of sites in CPD patients was significantly lower than controls (98.95± 15.08 Vs 110.50 ± 3.22, P=0.01). Data are shown in Table 4.

Results in Table 5 and Figure 1 show that the mean ± SD of salivary IL-6 concentration was not statistically different in CPD patients compared to controls (8.17 ±3.79 Vs 7.45± 1.51, P=0.4).

Table 1. Age and sex distribution of study groups.

Variables		Patients	Control	P-value
Mean age ± SD (Ys)		38.85 ± 5.30	40.52 ± 6.69	0.2
Gender	Male	20 (50%)	21 (52.5%)	0.8
	Female	20 (50%)	19 (47.5%)	

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.843

Table 2. PI and GI distribution of study groups

Variables	Patients	Control	P-value
Mean ± SD of PI	1.27± 0.34	0.60 ± 0.26	P=0.01
Mean ± SD of GI	1.49 ± 0.42	0.24 ± 0.14	P=0.01

Table 3. Distribution of bleeding on probing rate in study groups.

Bleeding on probing %	Patient group		Control group		P-value
	Score 1	Score 0	Score 1	Score 0	
No. of sites	3127	831	-	4420	0.001
Percentage	79.0%	20.99%	-	100	
Total (%)	3958 (100%)		4420(100%)		

Table 4. Distribution of PPD and CAL in CPD groups.

Variable	Patients	Control	P-value
Mean ± SD PPD (mm)	4.66 ± 0.50	-	-
Mean ± SD CAL (mm)	4.90 ± 1.30	-	-
Mean ± SD No. of teeth	24.72± 3.77	27.62± 0.80	0.01
Mean ± SD No. of site	98.95± 15.08	110.50 ± 3.22	0.01

Table 5. Salivary IL-6 concentration in study groups.

Groups	Mean	SD	SE of Mean	T-test	P-value
CPD Patients	8.17	3.79	0.893	0.75	0.4
Control	7.45	1.51	0.356		

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.843

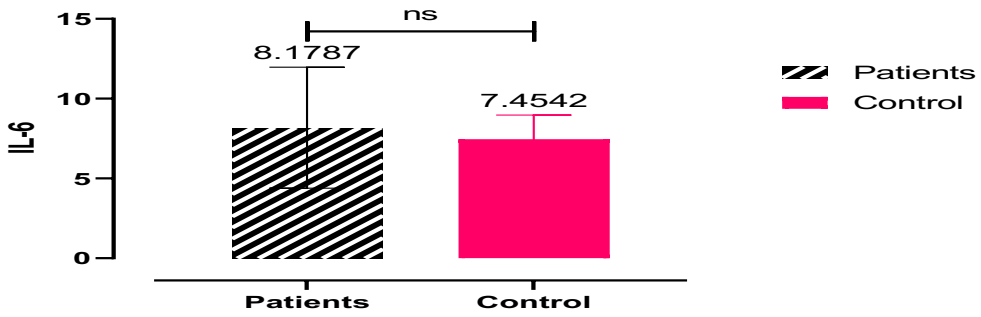

Figure 1. Salivary IL-6 levels (pg/ml) in study groups.

Results in Table 6 revealed the correlation of IL-6 levels in CPD patients with number of teeth and periodontal parameters, the r of No. of teeth, PDI, GI, BOP, PPD and CAL were 0.366, -370, -0.099, -0.281,-0.115 and -0.214 indicating insignificant negative correlation (P=0.135,0.131, P=0.695, P=0.259, P= 0.648 and P=0.394).

Concerning the molecular results, the qPCR technique showed that the mean ± SD fold of gene expression values of miRNA-146a in CPD patients was 1.79 ± 0.1 which was significantly higher than that of control individuals 1.08± 0.1 (P=0.001). Date was seen in Table 7 and Figure 2.

Data presented in Table 8 showed that the r values of the No. of teeth, PDI, GI, BOP, PPD and CAL were 0.190, -0.329, -0.069, -0.380, 0.143 and -0.217, respectively. All these parameters were not statistically different (P= 0.451, P= 0.183, P=0.785, P=0.120, P=0.571 and P=0.386, respectively).

Table 6. Pearson's correlation (r) of IL-6 levels in PCD patients with periodontal parameters.

Variables	r	P-value
No. of teeth	0.366	0.135
Periodontal Parameters		
PDI	-0.370	0.131
GI	-0.099	0.695
BOP	-0.281	0.259
PPD	-0.115	0.648
CAL	-0.214	0.394

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.843

Table 7. Mean ± SD of folds of miRNA146a expression in study groups.

Variables	Patient group	Control group
Means Ct of MiRNA146a	19.933	20.93
Means Ct of U6	14.4805	14.6677
ΔCt (Means Ct of MiRNA146a)	5.45333	6.2622
2-ΔCt	0.0228	0.0130
CPD group/ Control group	0.0228/0.0130	0.0130/0.0130
Fold of gene expression	1.756	1.00
Mean	1.7967	1.0876
SD	0.42824	0.46628
SE	0.10094	0.10990
P-value	0.001	

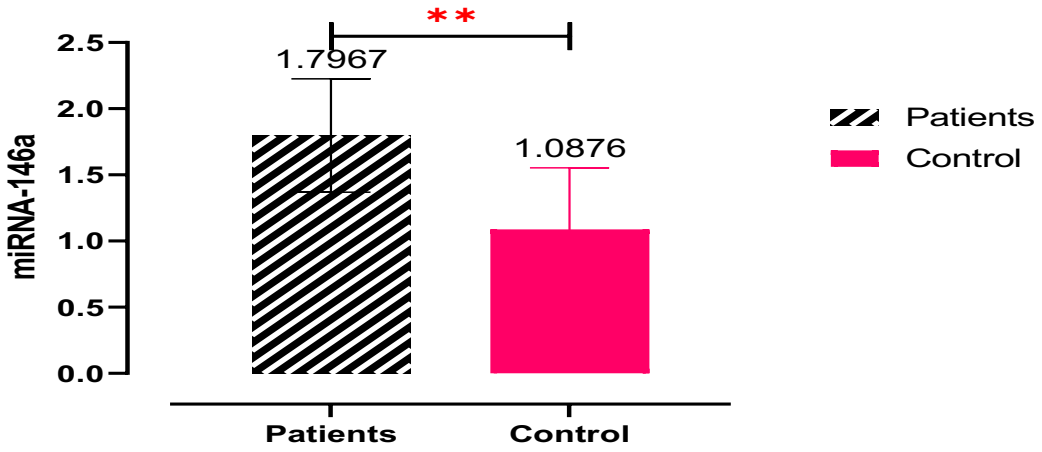

Figure 2. Mean folds of MiRNA146a expression in study groups.

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.843

Table 8. Person's correlation (r) of miRNA 146a expression in PCD patients with periodontal parameters.

Variables	r	P-value
No. of teeth	0.190	0.451 ^{NS}
Periodontal Parameters		
PDI	-0.329	0.183 ^{NS}
GI	-0.069	0.785 ^{NS}
BOP	-0.380	0.120 ^{NS}
PPD	0.143	0.571 ^{NS}
CAL	-0.217	0.386 ^{NS}

Discussion

Matched age and gender subjects among the study groups implies good sampling and reduced bias in our study. The results also found that there are significantly higher PI and GI in patients versus controls. Of note, the PI and GI are aimed to place more emphasis on the breakdown of periodontal tissues than on the inflammatory status of the gingiva, as it follows a scoring system ranging from 0 to 3, where 0 score reflects no plaque in the gingival area, while 3 score indicates abundance of soft matter within the gingival pocket and/or on the gingival margin and the adjacent tooth surface. The GI score starts with 0, which represents normal gingiva and a score of 3 denotes severe inflammation, marked redness and edema, ulceration, tendency to spontaneous bleeding [18]. These results are consistent with previous studies [19-21].

The current results found that the number of teeth and sites in healthy control are significantly higher than that in CPD patients. These results are logical and highly expected as the periodontitis eventually leads to teeth loss [22-25]. A factor that may explain the high rate of teeth loss among our patients is the bad phenomenon newly flourished in our community, the smoking of hookah or shisha among adolescents and young adults.

The low salivary IL-6 levels found in our patients may be the result of "paracetamol pills," a home remedy that is available in almost every Iraqi residency. Paracetamol is a nonsteroidal anti-inflammatory, analgesic, and antipyretic agent recommended as a first treatment for fever and mild to moderate pain including headache, toothache and muscle ache. Paracetamol may subside the inflammatory response and consequently lower IL-6 levels [26].

The molecular part of this study was the detection of salivary miRNA 146a. It was found that periodontitis can modulate the periodontal tissue levels of miRNAs [11] as well as miRNA

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.843

profile in saliva [12]. Overexpression or under expression of miRNAs can result in alterations in gene expression that might contribute to the development and/or progression of periodontitis [13]. Biofluids have been identified as best sources for miRNA biomarkers. Detection due to miRNAs can be easily isolated and identified using qPCR, miRNA sample collection is less invasive, and miRNAs are very stable in diverse biofluids [27]. Our result is consistent with previous studies suggesting the most widely explored miRNA in periodontal diseases was miRNA-146a [15,16]. The current results concluded that both IL-6 and miRNA 146a were elevated in CPD patients and closely correlated with periodontal parameters suggesting that these biomarkers can be used as a surrogate diagnostic and prognostic indicator of chronic periodontitis.

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Dentistry 3000
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