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Detection of Salivary Biomarkers Osteocalcin and Osteonectin of Bone Turnover among smokers Periodontitis Patients in Erbil City-Iraq

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ABSTRACT: This study aims to compare the periodontal health conditions between study and control groups, regarding clinical periodontal parameters (attachment loss, probing pocket depth, bleeding on probing, and plaque index), and also compare salivary biomarkers (Osteocalcin and Osteonectin) and their relationship. Eighty nine subjects participated in this study; they were distributed into three groups, sixty of them selected from patients seeking periodontal treatment, and their ages ranged between (20 and 50). The healthy group included (29) subjects of analogous ages to the study groups, with a clinically healthy periodontium state. The study groups consisted of (60) patients distributed equally to two groups (30) nonsmokers and (30) smokers with clinical signs of periodontitis. Our outcomes revealed a significant p. value ≤ 0.001 increase in parameters of periodontal in periodontitis group compared to the heathy group. There were no significant differences for all parameters between periodontitis groups, except bleeding on probing. While results of Osteonectin showed highly significant difference P. value ≤ 0.001 between the control and the smokers groups and similar results between the study groups. There was no significant difference between the control and nonsmoker groups. Statistical analysis indicated no significant association between periodontal parameters and salivary biomarkers. However, in the Osteocalcin group; the control group had a significantly different plaque index. Similarly in the osteonectin, the control group showed significant differences in bleeding on probing. The study concluded that smoking is a contributing factor that initiates bone recession. Osteocalcin and Osteonectin are potential biomarkers for the early diagnosis and prognosis of periodontal disease.

Keywords: Periodontitis, periodontal parameters, smoking, Osteocalcin, Osteonectin

1. INTRODUCTION

Periodontitis is a persistent inflammatory condition that damages the tissue that provides support to the teeth and eventually causes loss of the teeth [1]. A new Classification of periodontitis is categorized into periodontitis, necrotizing periodontitis and periodontitis resulting from systemic condition. Another one includes staging and grading. Staging evaluating the severity of the condition based on CAL. Periodontitis is also classified as localized, which has obvious features including (< 30% teeth) and generalized occurs without an obvious feature and including (≥30% teeth) [2]. Diagnosis of periodontitis is mainly relies on radiographic and clinical parameters of the periodontal such as plaque index (PLI), level of clinical attachment loss (CAL), periodontal pocket depth (PPD), bleeding on probing (BOP), and radiographic assessment of the volume of alveolar bone [3]. Periodontitis arises from the formation of symbiotic biofilms of subgingival plaque, which sustain an enhanced immune reaction within the gingival tissue. This creates a continuous cycle of host-bacteria interaction, where inflammation promotes further microbiome dysbiosis reversibly. If left untreated, the immune system response can result in irreversible damage to the alveolar bone and periodontal ligament (PDL). Consequently, loss of teeth occurs in susceptible individuals [4]. Risk factors are seen as components of the chain of events that lead to periodontitis, potentially raising an individual's susceptibility to the disease exposure.

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Such as cigarette smoking has been widely acknowledged as a contributing factor that can lead to the onset and progression of periodontal disease [5]. Biomarkers are biological markers that can substitute for ideally relevant endpoints or intermediate outcomes, which may be more challenging to observe directly [6]. Saliva is a highly abundant bodily fluid produced by the salivary glands and secreted into the mouth, lubricating the oral mucosa. It is a composite fluid of 99% water, along with minerals, the enzyme amylase, mucin, antibodies, proteins, inflammatory cells, and blood [7]. Salivary biomarkers have become promising tools for facilitating early detection, evaluating risks, and tracking the progression of periodontitis [8]. Osteocalcin (OC) is a key regulator in the process of bone turnover and is commonly identified as a biomarker associated with bone formation; as osteocalcin levels increase in bodily fluids, it means there is a disorder in bone turnover activity, which is detected during periodontitis [9]. In periodontitis, osteoclasts are drawn to the site of bone resorption by OC, which promotes their maturation into active osteoclasts. This shift in function might be associated with the disturbance of bone homeostasis owing to the increased resorption rate [10]. Various biomarkers linked to bone formation, resorption, and turnover, such as Osteonectin (ON) and OC, have been identified in both saliva and gingival crevicular fluid. These mediators are linked to the process of local bone loss and systemic conditions [11]. A deficiency in ON has been shown to result in reduced total collagen levels, with its production further diminished by periodontal disease. Therefore, ON appears to be a promising marker for monitoring the progression of the disease and evaluating the effectiveness of medical treatment [12]. The levels of both ON and N-propeptide alpha I type I collagen were significantly elevated in patient with periodontitis. However, ON was found to be a more sensitive marker for assessing the status of periodontitis compared to N-propeptide alpha I type I collagen [13].

2. MATERIAL AND METHOD

2.1 ETHICAL APPROVAL

The Ethics committee of Basic Dental Science, of the Faculty of Dentistry at Mosul University reviewed and approved the practical aspects of this research, adhering to guidelines for human studies under approval number UoM.Dent 23/53.The study's procedures were fully explained to the participants, and their written informed consent was obtained in compliance with these guidelines.

2.2 CLINICAL EVALUATION AND SAMPLE COLLECTION

The present study was accomplished in the Periodontal Clinical Unit in the KHANZAD DENTAL TEACHING CENTER, NADWA PRIVATE DENTAL CENTER, Military Hospital, and College of Dentistry of Hawler Medical University (Erbil City/Iraq)from July to December 2023. Eighty-nine subjects participated in the current study; sixty were selected from patients seeking periodontal treatment, and (29) were healthy individuals without periodontal disease; their ages ranged between (20 and 50). The clinical dental examinations for all participants were conducted chairside, assessing periodontal parameters such as bleeding on probing, clinical attachment loss, probing pocket depth, and plaque index using a periodontal probe World Health Organization (WHO). The depth of the pocket was calculated at four sites of each tooth. The space between the bases of the pocket to the cementoenamel junction represents CAL, whereas BOP was recorded using the procedure. Score 0= No bleeding after passing the periodontal probe, Score 1= bleeding occurring immediately or within 10 seconds after passing the periodontal probe [14]. PI was measured using various scores; score 0=, no plaque. Score 1= thin plaque deposit at the gingival margin with no visual observation, only by probe; Score 2= plaque is visible to the neck eyes also by passing probe; and Score 3= excessive accumulation of plaque exceeding the cervical third of the crown [15]. Following clinical examination, samples were taken from participants. Unstimulated saliva samples were obtained from 89 participants by allowing them to passively drool into a sterilized test tube. Saliva was centrifuged at 3000 rpm for 10min. The clear supernatant fraction was then separated and distributed in Eppendorf tubes and kept at -30˚C until the investigation.

2.3 SUBJECTS GROUPING AND SAMPLE SIZE

A total of 89 subjects participated in this study, 60 selected from patients seeking periodontal treatment, aged between (20 -50) years. The healthy control (group 1) consisted of 29 individuals of similar ages (20-50 years), with clinically healthy periodontium. The study groups were divided equally into two groups: 30 nonsmokers (group2) and 30 smokers (group3), both showing clinical signs such as plaque accumulation, gingival inflammation, PPD ≥ 4mm, and CAL≥ 2mm. The samples were examined by using the Sandwich Elisa technique.

2.3.1 INCLUSION CRITERIA

Individuals with periodontitis who have signs and symptoms of gum disease such as BOP, deep PPD, and CAL. Participants must have not received medications such as non-steroidal anti-inflammatory drugs, antibiotics, and dental procedures (scaling and polishing) within the last three months before examination. The participants in this study were male smokers and nonsmokers.

2.3.2 EXCLUSION CRITERIA

Females and individuals with systemic diseases that affect bone health, such as (diabetes and osteoporosis). Persons receiving (antiepileptic agents, corticosteroids, immunosuppressants and chemotherapeutics drugs) were excluded from enrollment in this study.

2.4 PROCEDURE FOR ANALYZING SAMPLES

Analysis was performed using the Human Osteocalcin ELISA Kit. USA.R&D System for Saliva. Human Osteonectin ELISA Kit's. R&D System for Saliva. The test principle applied in this study for both OC and ON was the Sandwich enzyme immunoassay kit, which ELISA performed. An antibody specific to human OC and ON was pre-coated onto the microtiter plate included in this kit, the same procedure was repeated. Saliva samples were placed into the assigned wells of the microtiter plate, and a biotin-labeled antibody was then added specific for OC and ON separately. Subsequently, Horseradish peroxidase conjugated to avidin was introduced into each well and incubated. The TMB substrate solution was then added, resulting in a color change in well containing OC or ON, the biotin-conjugated antibody, and the enzyme- conjugated avidin. The enzymatic reaction was stopped by adding sulfuric acid, and the color intensity was measured spectrophotometrically at a wavelength of 450 nm ± 10 nm. The concentrations of OC and ON in the samples were calculated by comparing their optical density values to a standard curve.

3. STATISTICAL ANALYSIS

Descriptive statistics were performed using IBM SPSS Statistics for Windows, version 25. The data were presented as mean values with standard deviations alongside the P value and test statistics. The significance of the difference between groups was assessed using the non-parametric (Kruskal-Wallis) test followed by Dunn's formula to identify which groups showed significant differences. Pearson correlation was employed to evaluate the strength and direction of the relationships between parameters across the three groups. The result of the Shapero-Wilk test in the current study revealed that the data do not have the normal distribution property, meaning that the assumption of normality of the data is not achieved since the P values were significant < 0.05 [16].

3.1 HYPOTHESES OF THE STUDY

According to the null hypotheses, there is no significant variation between the three groups in terms of periodontal parameter value (PLI, BOP, PPD, and CAL). There is no significant variation among three groups regarding salivary biomarkers value (Osteocalcin and Oteonectin).

4. RESULTS

4.1 COMPARISON OF PERIODONTAL PARAMETERS BETWEEN STUDY AND CONTROL GROUPS

The findings of the current study explained that the study groups exhibited significantly elevated in periodontal parameter levels compared to the control group with a highly significant p. value ≤ 0.001. However, no significant differences were observed in periodontal parameters among the group, except for BOP, which displayed a significant variations between periodontitis groups (nonsmokers and smokers) (Table 1) (Figure 1).

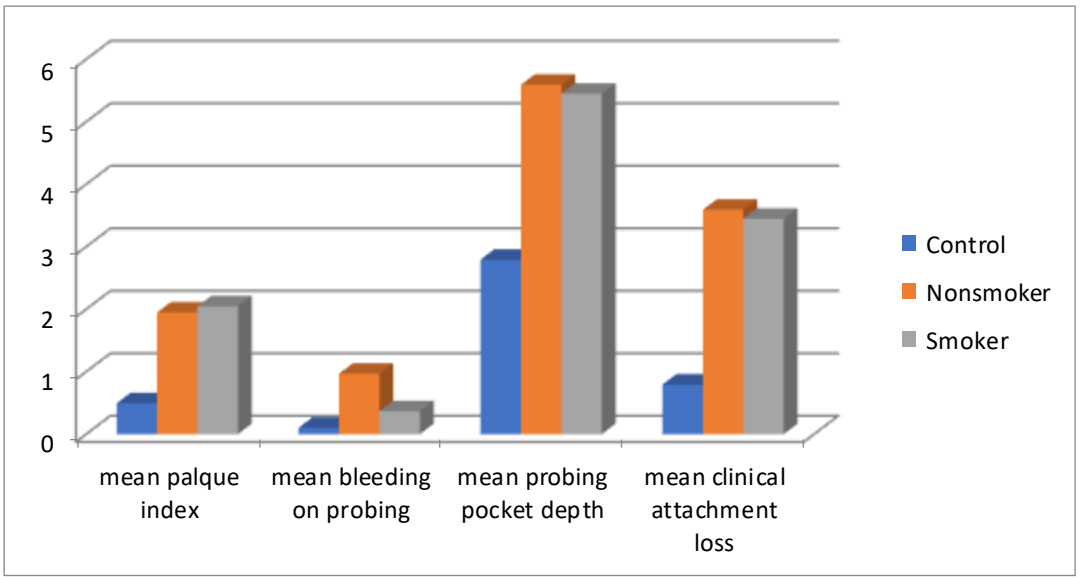

FIGURE 1. - Comparison of periodontal parameters between study and control groups

Table 1. - Comparison of periodontal parameters between study and control groups; significant difference existing between study and control groups at p.value ≤ 0.001.

Parameters	Groups	N	Mean	Std. Deviation	Kruskal - Wills H		
					Test value	P-value	Compare
mean PLI	Control	29	0.4924	0.3075	57.247	0.00 **	A
	Nonsmoker	30	1.9457	0.58354			B
	Smoker	30	2.0443	0.51194			B
mean BOP	Control	29	0.1014	0.06402	79.427	0.00 **	A
	Nonsmoker	30	0.9647	0.11717			B
	Smoker	30	0.367	0.13666			C
mean PPD	Control	29	2.7921	0.25633	59.352	0.00 **	A
	Nonsmoker	30	5.5897	0.51393			B
	Smoker	30	5.4473	0.78753			B
mean CAL	Control	29	0.7921	0.25633	59.352	0.00 **	A
	Nonsmoker	30	3.5897	0.51393			B
	Smoker	30	3.4473	0.78753			B

Data presented as mean, standard deviation, Test value, P-value and numbers. (A, B, C) represents compared groups; the identical letters (B, B) refer to two groups that are not significantly different for all parameters. And vice versa (A, B) (A, C) and (B, C) are referred to significant differences, p.value≤0.001. ** Highly significant difference.

4.2 COMPARISON OF SALIVARY BIOMARKERS BETWEEN STUDY AND CONTROL GROUPS

The analysis demonstrated that the mean OC biomarker level in the healthy group was (0.5778) showing a highly significant difference p.value ≤ 0.001), when compared to the periodontitis (nonsmoker and smoker) groups, whose mean values ranged from 2.6559 to 2.3864, respectively p. value ≤ 0.001. However, no statistically significant difference was detected between the (nonsmoker and smoker) periodontitis groups p. value > 0.05. Regarding the Osteonectin biomarker, the healthy group exhibited a mean value of (7.6307), with highly significant variation p. value ≤ 0.001), when compared with smokers periodontitis group which had a mean value (21.1838). In contrast, the difference between the healthy and nonsmoker periodontitis group (mean value 11.149) was not statistically significant. A highly significant difference p. value ≤ 0.001, was observed between nonsmoker and smoker periodontitis groups. Table (2) Figure (2)

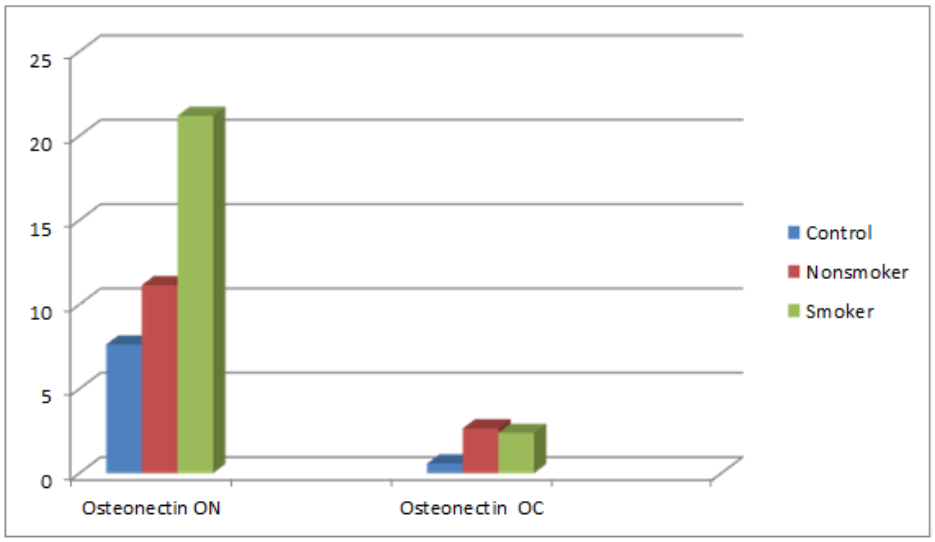

FIGURE 2. - Association of Salivary Biomarkers between study and control groups

Table 2. - Comparison of Salivary Biomarkers between study and control groups' significant difference existing between study and control groups only in ON no significant difference between control and nonsmokers at p. value ≤ 0.001

Parameters	Groups	N	Mean	Std. Deviation	Kruskal - Wills H		
					Test value	P-value	Compara
Osteocalcin	Control	29	0.5778	0.31078	58106	0.00 **	A
	Nonsmoker	30	2.6559	1.24519			B
	Smoker	30	2.3864	0.76249			B
Osteonectin	Control	29	7.6307	4.69048	51.079	0.00 **	A
	Nonsmoker	30	11.1492	5.17278			A
	Smoker	30	21.1838	0.91621			B

Data presented as mean, standard deviation, Test value, P-value and numbers. (A, B) represents compared groups; the identical letters (B, B) and (A, A) refer to two groups that are not significantly different for all parameters. And vice versa (A, B) is referred to significant differences, p≤ 0.001. ** Highly significant difference.

4.3 RELATIONSHIP BETWEEN SALIVARY BIOMARKER OC CONCERNING PERIODONTAL PARAMETERS

Table (3) demonstrates the correlation between Osteocalcin and periodontal parameters. While the correlation is not significant across all three groups, there is a notable correlation with PLI in the control group P.value = 0.001 (highly significant).

Table 3. - Relationship between OC and periodontal parameters among control group and study groups

Parameters	Osteocalcin					
	CONTROL		NOT SMOKER		SMOKER	
	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.
mean PLI	591**	0.001	-0.228	0.225	-0.165	0.385
mean BOP	-0.188	0.330	0.250	0.183	-0.283	0.129
mean PPD	0.272	0.154	0.018	0.926	0.298	0.110
mean CAL	0.272	0.154	0.018	0.926	0.298	0.110

Significant correlation in PLI in the control group (p= 0.001)

4.4 RELATIONSHIP BETWEEN SALIVARY BIOMARKERS ON CONCERNING PERIODONTAL PARAMETERS

Table (4) demonstrates the correlation between Osteonectin and periodontal parameters. While the correlation is not significant across all three groups, there is a notable significant correlation with mean BOP in the control group P= -0.035 (significant difference).

Table 4. - Relationship between ON and periodontal parameters among control and study groups

Parameters	Osteonectin					
	CONTROL		NOT SMOKER		SMOKER	
	Corr.	Sig.	Corr.	Sig.	Corr.	Sig.
mean PLI	-0.047	0.808	-0.044	0.818	0.148	0.434
mean BOP	-.393*	0.035	0.173	0.361	-0.245	0.191
mean PPD	-0.189	0.326	0.123	0.517	-0.036	0.851
mean CAL	-0.189	0.326	0.123	0.517	-0.036	0.851

Significant difference in BOP in control group

5. DISCUSSION

Periodontitis is a harmful condition driven by bacterial biofilm and autoimmune factors, affecting the soft and hard tissues of the periodontium [17]. This study focused on evaluating the main periodontal parameters such as (CAL, BOP, PLI and PPD). The statistics revealed that the average value for all measured parameters was higher in periodontitis groups compared to the healthy group, with statistically high significant variation ($P \leq 0.001$) between them. However, for BOP, there was no notable variation observed between healthy group and smoker's periodontitis group, in smokers individuals, this effect has been linked to reduced blood flow and gingival redness, resulting in decreased bleeding during periodontal probing, similar findings reported by Shukri and Zardawi, [18]. Among smoker individuals with periodontitis, higher values for the clinical periodontal parameters PL, PPD, and CAL were observed in smokers with periodontitis. The investigation of participants in the current research exhibited notable variation between the healthy group and the diseased groups. The average PLI in the periodontitis groups was higher than the healthy group. This could likely be attributed to individuals with periodontitis who were uncared for oral hygiene and did not regularly brush their teeth. Elevation of the mean PLI value reflects the impact of pathogens in the progression and development of periodontal disease. [19]. Considering the BOP level, our findings reveals that periodontitis patients exhibited pronounced periodontal disease activity. The evidence presented highlights a strong correlation between periodontitis and overall periodontal health. A study conducted by Bozyel et al., 2024 [20], demonstrated a higher significant variation in BOP between healthy individuals compared to periodontitis patients. In this study, the analysis showed no significant variations between the smokers' periodontitis group and the healthy group. This outcome coincides with a previous study done by Arruda et al. [21], who examined the clinical evaluation of gingival tissue in smoking individuals with chronic periodontitis revealed that smokers exhibit poorer periodontal health, characterized by reduced BOP and fewer teeth compared to nonsmokers and former smokers. The current study observed that the mean values for CAL and PPD were notably elevated in individuals with periodontitis compared to those in the healthy group, with significant differences evident between groups. These findings align with a study by Kumar et al. [22], who reported that smokers with chronic periodontitis displayed an elevation of pocket depth and clinical attachment loss levels compared to both nonsmokers with chronic periodontitis and healthy individuals. These outcomes are consistent with previous study findings by Velidandla et al. [23], who observed that cigarette smoking is linked to increase PPD and compromised immune function. While the differences in these parameters between periodontitis groups were not significant, this result also concurs with a study by Mahmood [24], which found no considerable difference in PPD and CAL between nonsmokers and smokers with periodontitis. Numerous studies have measured inflammatory markers in saliva, typically comparing individuals with healthy periodontium to those with diseased periodontium, assessing patients with various forms of periodontitis, or examining the impact of various periodontal treatments [25]. Osteocalcin is considered the primary protein in the bone extracellular matrix, containing glutamic acid residues. OC plays a major role in the process of bone turnover and is typically recognized as a biomarker for bone formation; as OC levels increase in bodily fluids, it means there is a disorder in bone turnover activity, which is detected during periodontitis [9]. Concerning OC results, the findings of the current research showed a distinct variation between the healthy and periodontitis groups, aligning with Bahrawy and Rauf [26] research, which found that OC levels in active periodontitis may elevate as a result of alveolar bone resorption. Another study by Joseph et al. [27] showed that salivary biomarker OC demonstrated the highest distinction between healthy controls and smokers with periodontitis. In the existing research, there was no significant variation between periodontitis groups. The results concord with the results performed by Nafarzadeh et al. [28], which found that OC levels did not exhibit any significant variation between nonsmokers and smokers with chronic periodontitis As far as we know, there are limited studies examining Osteonectin protein in the context of periodontal diseases [29]. In the present study, salivary ON was higher in smokers group compared to control and nonsmokers groups; however, a significant variation was identified between control and smoker groups. This outcome contrasts with earlier study, which suggested that ON has a strong capacity to distinguish the key characteristics of periodontal disease. Likewise, the study results indicated a statistically significant variation in salivary bone turnover biomarkers ON between healthy and periodontal compromised groups [12]. Also, our results is opposite to the outcomes of Joseph et al. [27], which concluded ON levels elevated progressively from healthy individuals to nonsmokers with periodontitis. The current study's outcome indicated a significant variation between nonsmokers and smokers group. The results of the correlation between OC and periodontal parameters, no significant relationship were detected between OC and periodontal parameters (CAL, PPD and BOP). There was a significant variation specifically with PLI in the control group. Our results are contrary to a former study by Joseph et al. [27], which reveals that salivary OC, ON, correlate positively with the periodontal parameters (BOP, PPD, and BL). Variations in OC levels among different research results and the present study might reveal the inability to distinguish between sites experiencing active attachment loss and those in a bone loss arrest state, where clinical indicators of periodontitis such as (clinical attachment loss, increased probing pocket depth, and bleeding on probing) are present but display no current activity [29]. The correlation analysis between ON and periodontal parameters revealed no significant variations between ON biomarkers and the three groups, except for BOP in the control group. This finding aligns with a previous study concerning BOP in the control group. However, there is a discrepancy when compared to the other three parameters (PPD, PLI, and CAL) from previous research, which found a positive correlation between salivary ON and (BOP, PPD, BL) [27].

6. CONCLUSION

Periodontitis is more prevalent among individuals with poor oral hygiene, particularly in smokers who do not adequately care for their oral health. The study determined that smoking is a risk factor that can trigger the onset of bone recession. OC and ON have been identified as potential biomarkers for the early detection and prognosis of periodontitis.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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