

Correlation between Periodontal Health Status and Salivary Matrix Metalloproteinase-9 Levels in Smoker and Non-Smoker Chronic Periodontitis Patients (A Comparative Study)

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ABSTRACT

Background: Periodontal diseases are inflammatory diseases affecting the supporting tissues of the teeth. One of the leading environmental factors that are closely related not only to the risk but also to the prognosis of periodontitis is smoking. This study aimed to evaluate the influence of smoking on periodontal health status and to measure the levels of matrix metalloproteinase-9 in smokers and nonsmokers chronic periodontitis patients, also it aimed to test the correlation between the levels of matrix metalloproteinase-9 and the clinical periodontal parameters.

Materials and Methods: Five milliliters samples of un-stimulated whole saliva and full-mouth clinical periodontal recordings (plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment level) were obtained from forty patients of two groups (non smokers with chronic periodontitis and smokers with chronic periodontitis). All subjects were systemically healthy males, with age range (35-50) years. Salivary matrix metalloproteinase-9 levels were analyzed by using Enzyme-linked Immunosorbent Assays.

Results: Statistical analysis revealed that probing pocket depth and clinical attachment level were higher in smokers than non smokers, while there were decreases in the numbers of bleeding sites in smoker when compared with non smoker subjects. Salivary matrix metalloproteinase-9 levels were significantly higher in smoker with chronic periodontitis patients than their non smoker counterparts.

Conclusion: Salivary matrix metalloproteinase-9, as a biomarker, could reflect the increased periodontal tissue destruction due to the smoking.

Keywords: Non-smokers, smokers, salivary matrix metalloproteinase-9, periodontal health status. (*J Bagh Coll Dentistry* 2016; 28(4):128-133)

INTRODUCTION

Chronic periodontitis is an infectious inflammatory disease characterized by the destruction of the tooth supporting structures (1). Periodontitis is a multifactorial irreversible and cumulative condition, initiated and propagated by bacteria and host factors (2). Several factors, including smoking, socioeconomic status and stress have been identified as potential risk factors for periodontitis (3).

Tobacco smoking is one of the most important risk factors associated with the destruction of the alveolar bone and loss of attachment in patients with periodontitis (4). Several studies on the relationship between periodontal diseases and tobacco use have consistently shown that the non smokers are two to six times less likely to develop periodontitis than smokers (5, 6). Smoking as an environmental factor has been suggested to interact with host cells and affect inflammatory responses to the microbial challenge (4). The effects of smoking include alterations in vascular function, monocyte /neutrophil activities, release of cytokine and inflammatory mediators and antibody production (7-10).

Biomarkers are defined as cellular, molecular, biochemical or genetic changes by which a normal, abnormal or simply biologic process can be noticed or monitored (11).

Matrix metalloproteinases (MMPs) represent a superfamily of proteases acting not only in physiological development and tissue remodeling, but also in pathological tissue destruction (12). Host cells are stimulated by pathogens in microbial dental plaque by increasing their MMP release, which is one of the indirect mechanisms of tissue destruction seen during periodontitis (13).

Matrix metalloproteinase-9, gelatinase B, is secreted mainly by neutrophils and it is capable of degrading denatured interstitial collagens, gelatins, laminin, elastin, fibronectin and collagens Type IV and Type VII (14). Generally smokers associated with higher GCF concentrations of MMP-9 than non-smokers (15).

SUBJECTS AND METHODS

Human subjects consist of 40 participants males with age range (35-50) years, attending department of periodontology at College of Dentistry / University of Baghdad. The sample population divided into two groups: 20 non smokers subjects with chronic periodontitis and 20 smokers subjects with chronic periodontitis.

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All subjects were in a good general health, with no history of systemic disease, no history of regular use of mouth washes and did not take medication (eg: anti inflammatory or antimicrobial therapy within the previous 3 month).

Saliva samples collection

Five milliliter sample of un-stimulated whole saliva was collected from each patient before the clinical periodontal examination.

The sample was collected after an individual was asked to rinse his mouth thoroughly with water to insure the removal of any possible debris or contaminating materials and waiting for 1-2 minutes for water clearance.

Salivary samples were collected at least 1 hour after the last meal and stored at -20 C° till being assessed for matrix metalloproteinase-9 levels.

Clinical periodontal examination

Clinical periodontal parameters included assessment of plaque index (PLI) (16), gingival index (GI) (17), bleeding on probing (BOP) (18), probing pocket depth (PPD) and clinical attachment level (CAL). We use scales for the measurements of PPD and CAL with the following scores {PPD: score (0) = 0-2 mm , score (1) >2-4 mm ,score (2) >4-6mm and score (3) >6mm }; {CAL: score (1)= 1-2 mm , score (2) >2-4 mm , score (3) >4-6 mm and score (4) >6 mm}.

Biochemical analysis

The biochemical analysis includes measuring the levels of matrix metalloproteinase-9 in saliva

by Enzyme-linked Immunosorbent Assays (ELISA), using (Quantikine R&D, USA) kit.

Statistical analyses

The study variables were statistically analyzed using Statistical Process for Social Science (SPSS version 20) by using mean, standard deviation, percentage, student t-test, chi-square test and Pearson's correlation coefficient, the level of significant was accepted at P ≤ 0.05 and highly significant when P ≤ 0.001.

RESULTS

A-Clinical periodontal parameters

The results of this study revealed that smoker and non smoker chronic periodontitis groups showed non significant differences in PLI and GI as shown in table (1).

The BOP results showed that smoker chronic periodontitis group had less numbers of sites with bleeding on probing than non smoker chronic periodontitis group, Chi-square test revealed highly significant difference between groups (P<0.001) as shown in table (2).

There were increasing in total numbers of PPD scores (2and 3) in smoker chronic periodontitis group compared to non smoker group, while scores (0 and 1) were decreased in smoker group. Chi-square test revealed significant difference in PPD between groups as shown in table (3).

The results of this study revealed increase in CAL (score 3 and 4) in smoker chronic periodontitis group when compared with non smoker group, Chi-square test showed significant difference between groups as shown in table (4).

Table 1: Descriptive statistics (mean±SD) and inter group comparison of (PLI, GI and MMP-9) between smokers chronic periodontitis and non smokers chronic periodontitis groups

	PLI Mean ± SD	t-test	P value	GI Mean ± SD	t-test	P value	MMP-9 (ng/ml) Mean ± SD	t-test	P value
Non smokers chronic periodontitis group	1.88±0.51	0.172	0.864	1.78±0.42	1.856	0.071	32.53±1.49	-	0.006
Smokers chronic periodontitis group	1.85±0.49			1.55±0.38			33.75±1.14		

Table 2: Number and percentage (in sites) of bleeding on probing scores and Chi-square test of smokers and non smokers chronic periodontitis groups

Scale	Non smokers chronic periodontitis group		Smokers chronic periodontitis group		X ²	P value
	No.	%	No.	%		
Score 0	483	25.6%	731	39.0%	77.1	< 0.001
Score 1	1405	74.4%	1145	61.0%		

Table 3: Number and percentage (in sites) of PPD scores and Chi-square test of smokers and non smokers chronic periodontitis groups

Scale	Non smokers chronic periodontitis group		Smokers chronic periodontitis group		X ²	P value
	No	%	No	%		
Score 0	80	4.2%	66	3.5%	14.9	0.002
Score 1	951	50.4%	842	44.9%		
Score 2	853	45.2%	962	51.3%		
Score 3	4	0.2%	6	0.3%		

Table 4: Number and percentage (in sites) of CAL scores and Chi-square test of smokers and nonsmokers chronic periodontitis groups

Scale	Non smokers chronic periodontitis group		Smokers chronic periodontitis group		X ²	P value
	No	%	No	%		
Score 1	70	3.71%	50	2.67%	8.77	0.032
Score 2	869	46.02%	830	44.24%		
Score 3	939	49.74%	975	51.97%		
Score 4	10	0.53%	21	1.12%		

B-Biochemical parameters

The mean and standard deviation of MMP-9 in non smokers chronic periodontitis group was (32.53± 1.49) ng/ml ,while it was (33.75± 1.14) ng /ml for smokers chronic periodontitis group , statistical analysis using student t-test showed significant difference between non smokers chronic periodontitis and smokers chronic periodontitis groups as shown in the table (1).

C-Correlation between clinical and biochemical parameters

There were no correlations between the levels of the salivary MMP-9 and PLI in smokers and

non-smokers chronic periodontitis groups, while there was significant positive correlation between GI and MMP-9 levels in non smokers group. Highly significant positive correlations were found between MMP-9 levels and BOP in smokers and non smokers groups as shown in table (5). There were highly significant positive correlations between MMP-9 levels and PPD scores (2 and 3) in smokers and non smokers groups, also there were highly significant positive correlations between MMP-9 levels and CAL scores (3 and 4) in smokers and nonsmokers groups as shown in table (6).

Table 5: correlations of (PLI, GI and BOP) and MMP-9 levels in smokers and non smokers chronic periodontitis groups

	Non smoker chronic periodontitis		Smoker chronic periodontitis	
	r	P value	r	P value
PLI	0.043	0.856	0.083	0.728
GI	0.453	0.031	0.381	0.125
BOP	0.807	< 0.001	0.652	0.001

Table 6: correlations of (PPD and CAL) scores and MMP-9 levels in smokers and non smokers chronic periodontitis groups

Groups	PPD				CAL				
	Score 0	Score 1	Score 2	Score 3	Score 1	Score 2	Score 3	Score 4	
Non smokers chronic periodontitis	r	0.402	0.447	0.954	0.897	0.174	0.201	0.660	0.829
	P	0.079	0.064	<0.001	<0.001	0.231	0.198	0.001	<0.001
Smokers chronic periodontitis	r	0.372	0.531	0.960	0.838	0.148	0.222	0.742	0.696
	P	0.106	0.082	<0.001	<0.001	0.267	0.174	<0.001	<0.001

DISCUSSION

In the present study non significant difference in PLI was found between smokers and non

smokers groups this result agrees with Calsina et al (19), while it disagrees with Mokeem et al (20).

The reason for this result is that the amount of plaque on the teeth surfaces is mainly depend on personal oral hygiene and frequency of teeth brushing rather than smoking status.

Although non significant difference was found in GI between smokers and non smokers groups, the results of this study showed that the non smokers group associated with higher GI than smokers group. This general increase in GI agrees with Preber and Bergstrom⁽²¹⁾, while it disagrees with Kolte et al⁽²²⁾.

According to this study non smokers chronic periodontitis group associated with more bleeding sites than smokers chronic periodontitis group with highly significant difference, this finding was in agreement with Calsina et al⁽¹⁹⁾ and in disagreement with Nassrawin⁽²³⁾.

The reasons for the suppression of the gingival inflammatory reaction and bleeding on probing in smokers are the products of tobacco smoke that interfere with the vascular inflammatory response. It was found that nicotine caused a severe drop in blood flow rates in spite of greatly increased pressure within the vascular system⁽²⁴⁾, also tobacco use was associated with reduced permeability of peripheral blood vessels⁽²⁵⁾. Vasoconstriction of peripheral vessels caused by smoking lead to inhibition of the vascular properties of inflammation such as redness, bleeding and exudation. Also smoking has been shown to affect oral polymorphonuclear (PMN) leukocytes, indicating a defect in PMN leukocytes -function^(26, 27). Thus, smoking seems to influence both cellular and vascular properties of the inflammatory reaction. The suppression influence of smoking on the inflammatory reaction might indicate an impairment of the defense mechanisms within the tissues and render them more susceptible to plaque infection.

Vasoconstriction of the gingival vessels of smokers might be attributed to the actions of nicotine-stimulated adrenaline and nor adrenaline on α_1 -adrenergic receptors. Although some evidences support this theory in animal models, the evidence that supports this hypothesis in humans is not founded⁽²⁸⁾.

According to results of this study, there were increase in PPD scores (2&3) in smokers chronic periodontitis group compared with non smokers chronic periodontitis group, while non smokers chronic periodontitis group had more sites of scores (0&1).

This general increase in PPD in smokers group compared with non-smokers group was in agreement with many studies^(29, 30) and it was in disagreement with Kubota et al⁽³¹⁾. Studies had shown that nicotine suppresses mineralized

nodule formation⁽³²⁾. Additionally, osteoclast differentiation is enhanced by nicotine through macrophage colony-stimulating factor and prostaglandin E2 production, which are produced by nicotine-treated osteoblasts⁽³³⁾.

It was found that nicotine concentrations of gingival crevicular fluid could be nearly 300 times more than that of plasma concentrations in smokers⁽³⁴⁾, also nicotine bound to root surface and in vitro studies showed that it could be stored and released from periodontal fibroblasts. Nicotine could inhibit fibroblast attachment and integrin expression, fibronectin and collagen production and increased fibroblast collagenase activity⁽³⁵⁾.

According to the results of this study, there were increase in CAL scores (3 and 4) in smokers chronic periodontitis group when compared with non smokers chronic periodontitis group. This general increase in CAL in smokers group compared with non-smokers group was in agreement with Mokeem et al⁽²⁰⁾; Nassrawin⁽²³⁾. The reason for this increased CAL could be derived from the same explanations of increased PPD in smokers which were mentioned previously as both of them could reflect the progress and severity of periodontal tissue destruction.

This study showed that MMP-9 levels were higher in smoker chronic periodontitis group than non smoker chronic periodontitis group, this finding was in agreement with Victor et al⁽¹⁵⁾. This increase in MMP-9 concentrations of smokers also shown at systemic levels, as plasma MMP-9 levels of smokers were 6.45 times higher than that of non-smokers⁽³⁶⁾.

Cigarette smoke contains high concentrations of reactive oxygen species (ROS), ROS can activate proinflammatory signaling pathways and induce bone resorption^(37, 38).

Studies had shown that nicotine shift neutrophil function towards destructive activities⁽³⁹⁾, and induce the expression of MMPs in osteoblasts⁽⁴⁰⁾. Smoking also change the balance between tissue inhibitor of matrix metalloproteinase (TIMP-1) and MMPs, as MMP-9/TIMP-1 ratios were higher in smoker chronic periodontitis than non-smoker chronic periodontitis group⁽⁴¹⁾.

Additionally, this study found that the MMP-9 levels directly correlated with the clinical periodontal parameters (BOP, PPD and CAL). These results were in agreement with Rai et al⁽⁴²⁾; Isaza-Guzman et al⁽⁴³⁾. The mean MMP-9 levels in pocket sites were higher when the test site had a pocket ≥ 4 mm than in sites with a PPD of < 4 mm⁽⁴⁴⁾.

Consequently, MMP-9 concentrations of saliva could reflect the degree of periodontal inflammation and tissue breakdown.

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