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Original Article

The influence of smoking on the levels of matrix metalloproteinase-8 and periodontal parameters in smoker and nonsmoker patients with chronic periodontitis: A clinicobiochemical study

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ABSTRACT

Aims: Even though worldwide evidences tend to prove that smoking adversely influences periodontal health, there are few studies demonstrating the effect of levels of salivary matrix metalloproteinase-8 (MMP-8) in smokers. This study aimed to compare and quantify the levels of MMP-8 in smokers and also to evaluate the effect of smoking on periodontal indices in smokers and nonsmokers with chronic periodontitis.

Methods: A total number of 60 subjects were selected for the study and were divided into three groups: group I, healthy nonsmoking subjects; group II, nonsmoking patients with chronic periodontitis; group III, smoking patients with chronic periodontitis. Each group consisted of 20 subjects. Saliva sample was collected for the estimation of MMP-8 by enzyme-linked immunosorbent assay (ELISA) method using Quantikine human total MMP-8 immunoassay kit.

Results: The levels of the salivary MMP-8 of group III was highest followed by group II and group I, the least. The other periodontal indices, viz. plaque index (PI), probing pocket depth (PPD), clinical attachment level (CAL), were highest for group III, except for gingival index (GI).

Conclusion: This study suggests that MMP-8 is involved in periodontal destruction associated with smoking. Additionally, smoking exerts disastrous effects on immune response and can affect the pathogenesis of disease; hence, smoking results in increased severity of periodontal destruction.

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1. Introduction

Smoking is now considered as one of the most important risk factors in the development and progression of periodontal disease.^{1,2} Smoking-induced changes in neutrophil reactive oxygen species (ROS) within periodontal tissues play a role in increasing oxidative stress and reducing innate immune responses to periodontal pathogens and their products, thereby contributing to periodontal tissue damage and disease progression.³ It is also associated with increase in pocket depths, alveolar bone loss and increased rate of tooth loss.^{4,5} Cigarette smoking affects neutrophil function which are major source of matrix metalloproteinase-8 (MMP-8).^{6,7} Cigarette smoke contains a mixture of chemicals having carcinogenic potential, as well as various stable and free radicals species, and ROS that causes cellular oxidative stress, which is a factor for many smoking-related diseases. This smoke-induced oxidative stress has the potential to alter the MMP activity. It may increase the MMP expression via activation of inflammatory transcription factors.⁸

Human MMPs are a group of 23 structurally related but genetically distinct endopeptidase enzymes that cleaves the internal peptide bonds of proteins. They have the capacity to degrade practically all extracellular matrix, basement membrane matrices, and their components.⁹ There are several groups of MMPs: Collagenase (MMPs 1, 8, 13), Gelatinase (MMPs 2, 9), Stromelysin (MMPs 3, 10, 11), and membrane-associated MMPs. Among these, MMP-8 (collagenase-2) has been considered as a key marker in chronic periodontitis, and is synthesized by differentiating granulocytes in the bone marrow and stored in specific granules of circulating neutrophils. It is detectable in saliva and gingival crevicular fluid (GCF).¹⁰ It exhibits a unique ability to decompose Type I and III collagens,¹¹ and is highly correlated with bleeding on probing, attachment loss, and probing pocket depth (PPD). In healthy condition, the periodontal ligament apparatus is shielded from MMP-mediated proteolytic attack by tissue inhibitors of metalloproteinase (TIMPs). In pathologic conditions like chronic periodontitis, the amount of TIMP is low and thus inadequate to inactivate the elevated levels of MMPs, leading to aberrant connective tissue destruction. Hence, evaluating the level of MMP-8 will enable us to understand how tobacco smoke-induced alteration in MMP-8 levels may contribute to the increased susceptibility of periodontitis in smokers.

2. Methods

2.1. Patient selection

The present study was conducted in the Department of Periodontology of Dr. Z.A Dental College, Aligarh Muslim University, Aligarh, India. This study was approved by the Institutional Review Board. All patients were informed about the study and informed consent forms were signed in full accordance with the declaration of Helsinki.

2.2. Groups

A total of 60 study participants, in the age range of 35–55 years and having at least 20 teeth were enrolled into the study and were further divided into three groups: group I: healthy nonsmoking subjects, group II: nonsmoking patients with chronic periodontitis, and group III: smoking patients with chronic periodontitis.

2.3. Inclusion criteria

Group I: Periodontally and systemically healthy individuals with no apparent signs of clinical inflammation.

Group II: Subjects who never smoked and were systemically healthy but clinically diagnosed with moderate to severe chronic periodontitis (e.g. at least two or more interproximal sites with clinical attachment level (CAL) of ≥ 4 mm, or two or more interproximal sites with PPD of ≥ 5 mm, not on the same tooth).¹²

Group III: Subjects who had smoked ≥ 1 pack/day for at least past 10 years and were systemically healthy but clinically diagnosed with moderate to severe chronic periodontitis (e.g. at least two or more interproximal sites with CAL of ≥ 4 mm, or two or more interproximal sites with PPD of ≥ 5 mm, not on the same tooth).

3. Exclusion criteria

Pregnant and lactating women, patients with acute or chronic medical disorders, patients under any medication for the past 3 months, patients who had undergone any surgery in past 6 months, and subjects undergoing orthodontic treatment were not included in the study.

3.1. Periodontal assessment

Periodontal disease status of all patients was evaluated by the measurement of gingival index (GI),¹³ plaque index (PI),¹⁴ pocket probing depth, and CAL, using the same periodontal probe (University of North Carolina-15 probe (UNC-15), Hu-Freidy's, USA) and conducted by the same examiner to avoid bias. Clinical periodontal examination was performed by a trained and calibrated investigator who was masked to groups. The overall k for intra-examiner reliability was 0.75.

3.2. Method of collection of saliva sample

The clinical parameters described above were recorded and saliva samples necessary for the estimation of MMP-8 were taken from all groups. Subjects were told not to eat or drink 2 h prior to the examination. They were then asked to rinse and 3 ml of unstimulated whole expectorated saliva was collected from each subject and placed into sterile 5 ml saliva collecting tubes according to method described by Navazesh.¹⁵ The collected saliva was centrifuged immediately to remove cell debris ($1000 \times g$ for 10 min at 4°C). Then the supernatant was removed and sample was stored in small aliquots at -80°C until analysis. Salivary MMP-8 levels were estimated by using Quantikine human total MMP-8 immunoassay kit using

Table 1 – Demographic data and clinical parameters (mean $\pm$ SD) of study groups.

Parameters	Group I (n = 20)	Group II (n = 20)	Group III (n = 20)	P value
Age (years)	43.30 $\pm$ 8.64	42.80 $\pm$ 8.02	44.20 $\pm$ 7.40	0.856*
Gender				
Male	10 (50%)	11 (55%)	14 (70%)	0.606*
Female	10 (50%)	9 (45%)	6 (30%)	
GI	0.54 $\pm$ 0.11	2.38 $\pm$.39	1.62 $\pm$ 0.11	<0.001***
PI	0.57 $\pm$ 0.10	2.22 $\pm$ 0.20	2.45 $\pm$ 0.24	<0.001***
PPD	1.94 $\pm$ 0.30	5.26 $\pm$ 0.22	5.91 $\pm$ 0.23	<0.001***
CAL	0.60 $\pm$ 0.06	4.60 $\pm$ 0.30	5.33 $\pm$ 0.39	<0.001***

Data are expressed as mean $\pm$ standard deviation.

Group I (control), group II (nonsmokers with chronic periodontitis), group III (smokers with chronic periodontitis). GI: Gingival Index; PI: Plaque Index; PPD: Probing pocket depth; CAL: Clinical attachment level.

* Not significant.

*** Highly significant.

enzyme-linked immunosorbent assay (ELISA) method, provided by R and D Systems (Minneapolis, USA). Absorbance was read at 450 nm, with correction wavelength set at 540 or 570 nm.

3.3. Statistical analysis

Continuous data were summarized as mean $\pm$ standard deviation (mean $\pm$ SD) while discrete (categorical) in %. Continuous variables were compared by one-way analysis of variance (ANOVA), and the significance of mean difference between the groups was done by Tukey's post-hoc test. Categorical variables were compared by chi-square (χ^2) test. Pearson correlation analysis was done to assess correlation between MMP-8 and periodontal status. A two-sided ($\alpha = 2$) $P < 0.05$ was considered statistically significant. All analyses were performed on STATISTICA (Windows version 6.0).

4. Results

4.1. Clinical observation

The mean values for the clinical parameters and demographic data of all the three groups are given in Table 1. No significant difference between the mean age was found among the three groups ($P > 0.001$). Similarly no significant difference was found in the proportion of genders (M/F) between the three groups ($P > 0.001$). A comparison among all the three groups showed a significant difference in periodontal parameters ($P < 0.001$). The mean PI, PPD, CAL values of groups II and III were significantly higher than those of group I ($P < 0.001$), with the highest value in group III. However, the mean GI values of groups II and III were statistically higher than that of group I ($P < 0.001$), with the highest value in group II.

MMP-8 levels, Salivary MMP-8 levels of group II (354.83 $\pm$ 29.91) and III (459.16 $\pm$ 24.30) were significantly higher than group I (174.17 $\pm$ 22.40), with the highest being in group III. Further, Tukey test revealed significant difference ($P < 0.001$) among all three groups with respect to MMP-8 levels (Fig. 1). The data of Table 2 revealed a significant positive correlation (r) between MMP-8 and periodontal parameters in entire sample.

Fig. 1 – Salivary MMP-8 levels of three groups. Group I: control; group II: nonsmoking patients with chronic periodontitis; group III: smoking patients with chronic periodontitis. In group III, the levels of MMP-8 were significantly increased as compared to group II and group I. a) Statistically significant difference as compared between groups I and II ($P < 0.001$). b) Statistically significant difference as compared between groups II and III ($P < 0.001$).

Table 2 – Correlation between salivary MMP-8 levels and periodontal parameters.

Parameters	Correlation (r) value	P value*
GI	0.65	0.000
PI	0.93	0.000
PPD	0.95	0.000
CAL	0.94	0.000

GI: Gingival Index; PI: Plaque Index; PPD: Probing pocket depth; CAL: Clinical attachment level.

* Correlation is significant at the 0.01 level (2 tailed).

5. Discussion

MMP-8 has been considered as a key biomarker in chronic periodontitis. It is a major collagenolytic metalloproteinase in gingival tissues and oral fluids and its elevated level is associated with the severity of periodontal inflammation. Until now few studies have reported the levels of MMP-8 in

chronic periodontitis patients to be associated with smoking. In this study the quantitative analysis of MMP-8 showed that MMP-8 levels were significantly increased in smokers (group III) as compared to non-smokers (group II), although both having generalized chronic periodontitis. Some sort of doable justification pertaining to this could be that the harmful effects of smoking may interfere with vascular and inflammatory phenomena, as well as by impairing the granulocyte function, which reacts to microbial challenges by delivering more amount of serine proteases, elastase, and MMP-8, which are related to degradation of periodontal tissue.⁵ The results of the present study are in agreement with findings of other studies in which Liu et al.¹⁶ and Soder¹⁷ found an increase in salivary MMP-8 level in smokers with chronic periodontitis when compared to control. In contrast, one report described by Liede et al.¹⁸ who suggested that smoking may significantly lower both general proteolytic activity and MMP-8 level in saliva. As we know, periodontal tissue breakdown is higher in smokers; it is somewhat surprising that smokers showed lower salivary proteolytic enzyme activity than nonsmokers. This can be explained by the fact that the reactive oxygen species present in cigarette smoke can not only activate latent pro-MMPs but also inactivate and fragment MMP-8.^{19,20} Furthermore, in the present study, the MMP-8 showed a positive correlation with periodontal parameters, which is in accordance with the study by Soder.¹⁷ This indicates that periodontal disease severity increase with the increased levels of MMP-8.

There is substantial evidence to support considering smoking as a most important environmental factor which is associated with increased periodontal destruction. Such an increase in the risk of periodontitis could be due to the effect of tobacco on the host immune system by restraining chemotactic and phagocytic functions of polymorphonuclear leukocytes, diminishing antibody production, and predisposing periodontal tissue to infections.²¹ In the present study, it was found that the value of pocket depth was highest for smokers as compared to nonsmokers, which is in accordance with the findings by Linden and Mullally²² that the percentage of sites with probing depths more than 4 mm was more than double in smokers (15%) compared with nonsmokers. Haffajee and Socransky²³ in a study similar to ours reached the same conclusion in respect to pocket depth in nonsmokers. In addition, the result of this study also showed that greater attachment loss was present in smokers as compared to nonsmokers, which was in agreement with the Grossi et al.²⁴ who found strong association between smoking and attachment loss.

Cigarette smoking has clearly been implicated as contributing to periodontal breakdown and thus responsible for increased severity of periodontal destruction. Moreover, our findings are consistent with prior studies by Johnson and Bain²⁵ and Albandar²⁶ who reported an increased prevalence and severity of periodontitis, greater marginal bone loss, more severe attachment and periodontal bone loss, deeper periodontal pockets, and more teeth with furcation involvements among smokers. The strong association between smoking and periodontitis in this study is consistent with the finding of Bergstrom et al.²⁷ and Kerdvongbundit and Wikesjo²⁸ who reported that mean gingival recession, pocket depth, and loss of attachment were significantly higher among smokers.

At the same time, the present study also showed that GI was lower in smokers as compared to nonsmokers, which is in accordance with Feldman et al.²⁹ who reported that smokers with periodontal disease had less gingival bleeding and inflammation as compared to nonsmokers with periodontitis. This might be reinforced by the point that one of the byproducts of cigarette smoke and nicotine exerts local vasoconstriction, and heavy gingival keratinization causes reduced blood flow, edema, and therefore mask the early signs of periodontal disease by decreasing gingival inflammation, bleeding, and erythema. One more study was conducted by Danielsen et al.³⁰ reported that smokers exhibited less gingival bleeding and inflammation than nonsmokers.

Whilst the finding of the study could be applied in most instances, there were some limitations of this study, such as small sample size. Thus, studies with larger sample size should be performed so that various associated risk factors could be studied in detail. Also this is a cross-sectional study, we could not determine whether the exposure to smoking lead the onset of disease, nor would be able to differentiate periodontal sites with active disease from those with long standing periodontal disease; however, in the light of the existing evidence, it can be concluded that the more destructive nature of periodontal disease in smokers leads to an increase in salivary MMP-8 level and the elevated levels might be useful in monitoring periodontal disease in smokers. Therefore, smoking may interfere with the levels of MMP-8 and should be considered when using saliva for diagnosis. Additionally, the current study shows that smoking is a major environmental risk factor that exerts a negative effect on periodontal tissues of smokers, which contribute to an enhanced susceptibility to periodontitis.

6. Conclusion

To conclude, the finding of increased periodontal levels of MMP-8 in smokers, when compared to nonsmokers, gives additionally the research of which oral health experts ought to be prompted to stay active in assisting to scale back the burden associated with cigarette smoking within their areas by simply promoting, counseling, and facilitating smoking cessation among their patients.

Conflicts of interest

The authors have none to declare.

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