Research Article

Assessment of Salivary Matrix Metalloproteinase-8 level among cigarette smokers with and without chronic periodontitis (A Comparative Study)

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Abstract

Chronic periodontitis is an infectious inflammatory disease initiated and propagated by bacteria and host factors and characterized by the destruction of the tooth supporting structures. Smoking is considered as an environmental factor that act together with host cells and affect inflammatory responses to the microbial challenge. Increased salivary MMP-8 levels are associated with progressive loss of attachment in periodontitis.

The present study was conducted to investigate the association of the levels of salivary MMP-8 with commonly used periodontal clinical indices of groups of chronic periodontitis patients accounting for their smoking habits.

A convenience sample of eighty-one subjects (53 males and 28 females) was recruited for this study. The mean age of the study population was (36.6±9.2) years. The sample population was categorized into three groups: 26 non-smoker subjects with chronic periodontitis, 26 smoker subjects with chronic periodontitis and 29 periodontally healthy subjects. The clinical periodontal parameters were assessed at six sites and it included Plaque Index (PI), Gingival Index (GI), Pocket Depth (PD) and clinical attachment loss (CAL).

MMP-8 level was analyzed in salivary samples using Biolegend’s ELISA MAX™ standard set which is a quantitative sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The MMP-8 level expressed in nano-gram per milliliter (ng/ml).

The results of this study showed significant differences in clinical periodontal parameters among the three groups (PI, PD and CAL) with p values of (0.001, 0.02, 0.001 respectively). Statistical analysis using one-way ANOVA showed a significant difference between the three groups in salivary MMP-8 levels with the highest value among the smokers with chronic periodontitis. On the other hands, no significant correlations were detected between the levels of the salivary MMP-8 and the other clinical periodontal parameters (GI, PD and CAL) among each of the study groups.

The present study clearly shows a profound effect of smoking on salivary MMP-8 in chronic periodontitis subjects in comparison to non-smokers and periodontally healthy subjects.

BACKGROUND

Chronic periodontitis is an infectious inflammatory disease initiated by bacteria and propagated by host immune response, the disease is characterized by the destruction of the tooth supporting structures including, connective tissues and alveolar bone [1,2].

Although microorganisms (mainly anaerobic bacteria) are considered as instigating agents, the disease progression is influenced by the host response together with environmental and behavioral factors [3].

The mechanism by which the disease is progressed encompasses a complex of interacting molecular pathway of a set of inflammatory mediators such as cytokines, growth factors, reactive oxygen species, matrix metalloproteinase (MMPs), and their inhibitors and regulators [4].

Matrix metalloproteinases are a large family of calcium dependent zinc containing endopeptidases. MMPs are considered as essential host factors that contribute in periodontal diseases pathogenesis. They control collagen and extracellular matrix (ECM) degradation of periodontal tissues. The MMPs family encompasses around 25 members that are basically classified into six groups, which are contribute innumerous physiological and pathological conditions [5,6].

The balance of MMP activities is controlled by endogenous inhibitors such as tissue inhibitors of metalloproteinase (TIMP)

and any discrepancy between MMP and TIMP levels plays an important role in the periodontal disease progression [7].

Inflammatory destruction of periodontal attachment apparatus results from the degradation action on collagen fibers that is mediated by MMPs which are secreted by the resident cells of PDL in response to inflammatory stimuli. Previous studies revealed that the MMPs most commonly involved in periodontal tissue destruction are MMP-8, MMP 9, MMP-13, and MMP-14 [8,9].

MMP-8, also known as collagenase-2 or neutrophil collagenase, has been linked to inflammatory conditions. It is secreted mainly by neutrophils [10]. However, it can also be expressed by gingival fibroblasts, endothelial cells, epithelial cells, plasma cells, macrophages, and bone cells [11,12].

Increased salivary MMP-8 levels are associated with progressive loss of attachment in periodontitis [13].

During the past 25 years, studies have focused on the role of cigarette consumption on oral health problems including periodontal disease. Smoking is considered as a major risk factor in the prevalence, extent and severity of periodontal diseases [14-16]. It is also considered as an environmental factor that acts together with host cells in mediating the inflammatory responses to the microbial challenge [17].

The suggested mechanism by which smoking affects the periodontal tissues includes alterations in vascular function, monocyte/neutrophil activities, release of cytokine and inflammatory mediators and antibody production [18].

However, the effect of smoking on salivary MMP-8 in respect to their association with the periodontal health is not well elucidated. Many studies reported lower levels of MMP-8 concentrations in adults who smoke than in non-smokers. On the other hand, similar MMP-8 concentrations were expressed in sites with progressive periodontal disease irrespective of smoking status [19-21].

Though recent evidence indicates that smoking is a risk and modifier factor of periodontal diseases, conflicting results obtained from previous studies considering the level of MMP-8 in regards to the attachment loss and periodontal destruction among smokers with chronic periodontitis [21,22].

The present study aimed to investigate the association of the levels of salivary MMP-8 with commonly used periodontal clinical parameters of groups of chronic periodontitis patients accounting for their smoking habits.

**MATERIAL AND METHODS**

**Study Subjects and Clinical Examination**

This cross-sectional study was conducted at the Periodontal Department by Faculty of Dentistry, University of Khartoum and Periodontal Department at Khartoum Dental Teaching Hospital between April 2017 and October 2017.

A convenient sample of eighty-one subjects (53 males and 28 females) was recruited for this study. The mean age of the study population was (36.6±9.2) years.

The study protocol was approved by the research ethics committee of the Faculty of Medicine, University of Khartoum (HREC assigned number FM/DO/EC), according to the principles of the Helsinki Declaration.

The sample population was categorized into three groups: 26 non-smoker subjects with chronic periodontitis, 26 smoker subjects with chronic periodontitis and 29 periodontally healthy subjects.

All subjects who participated in the study have no history of systemic disease and did not take medication such as anti-inflammatory or antimicrobial therapy within the previous 3 months.

The Non-smokers Chronic periodontitis group consisted of 12 males and 14 females with mean age (40.1±10.9 years). CP patients were diagnosed according to the clinical criteria listed in the consensus report of the World Workshop in Periodontitis (Armitage, 1999) [23].

The group of periodontally healthy subjects comprised of 14 females and 15 males with a mean age of 30.9±6.9 years. They had at least 20 teeth, with sulcus depth <3 mm and CAL, ≤1 mm. Additionally, the group should not exhibit overt signs of clinical inflammation.

**Clinical periodontal examination**

All participants assigned a written informed consent prior to participation in the study.

Before clinical examination, a structured questionnaire was completed by each participant to record their general health and health habits, such as smoking, tooth brushing.

The clinical periodontal parameters were assessed at six sites/tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual locations) and included Plaque Index Sillness and Loe (PI) [24], Gingival Index Loe and Sillness (GI) [25].

Pocket Depth (PD), Clinical Attachment Loss (CAL) using Michigan O probe with William’s marking, periodontal examination was conducted by the one examiner to avoid bias. The examiner was trained and calibrated, with kappa value of 0.8.

**Protocol used for salivary samples collection and Analyses of Salivary MMP-8 Levels**

Saliva samples were obtained from all patients between 9 and 11 a.m. at the day of the intraoral examination. Unstimulated whole saliva was collected in sterile collection tubes from each subject according to the method described by Navazesh [26] as follow:

1. The patient was asked to refrain from intake of any food or beverages (water is exempted) one hour before the saliva collection.
2. The patient was asked to rinse his/her mouth with distilled water and then to relax for five minutes.
3. Then the patient was asked to swallow to void the mouth from saliva.
4. After that the patient was asked to sit in an upright position and lean his/her head forward over the collection tube and allow saliva to drain into the tube for five minutes and then spit any remaining saliva into the tubes quickly.

**Analyses of Salivary MMP-8 Levels**

Collected samples were centrifuged immediately to remove cell debris (1000g for 10min at 48°C). Then the supernatant was removed and sample was stored at −80°C until the time of analysis.

For assessment of MMP-8 levels (ng/ml), the salivary samples were analyzed by Biolegend’s ELISA MAX™.

The LEGEND MAX™ Human MMP-8 Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit includes a 96-well strip plate that is pre-coated with a mouse monoclonal anti-human MMP-8 antibody. The Detection Antibody is a biotinylated mouse monoclonal anti-human MMP-8 antibody. This kit is specifically designed to accurately measure MMP-8. The detection limits and inter-assay coefficients of variation were 0.08 ng/mL and 7.1 % for MMP-8.

This assay has high sensitivity and excellent specificity for detection of Matrix Metalloproteinase 8 (MMP8). No significant cross-reactivity or interference between Matrix Metalloproteinase 8 (MMP8) and analogues was observed.

**Statistical analysis**

The data of this study was presented in frequencies, tables and graphs. Continuous variables were summarized as mean ± standard deviation and were compared by using one-way analysis of variance (ANOVA), and the significance of mean difference between the groups was done by Bonferroni post-hoc test.

Pearson correlation analysis was done to assess correlation between MMP-8 and clinical periodontal parameters.

P<0.05 was considered statistically significant. All analyses were done by using Statistical Package for the Social Sciences® (SPSS), computer software version 22 was used for analysis of the data obtained.

**RESULTS**

**Clinical measurements of the periodontal parameters**

When a comparison was made among the three groups in regards to their clinical periodontal parameters, the results showed significant differences in (PI, PD and CAL) with p values of (0.001, 0.02, 0.001 respectively). The mean PI, PPD, CAL values of the non-smoker chronic periodontitis and the smoker periodontitis groups were significantly higher than those of periodontally healthy group, with the highest value among the group of smokers with chronic periodontitis.

Conversely, no statistically significant difference was found in the mean GI values among the three groups (P=0.05) (Table 1).

**Salivary MMP-8 levels**

The mean and standard deviation of salivary MMP-8 in non-smokers’ chronic periodontitis group was (0.418± 0.66) ng/ml, in the second group which included the smoker chronic periodontitis subjects, the mean and standard deviation was found to be (0.785± 1.068), where as in the third group of periodontally healthy subjects a mean and standard deviation of (0.137±0.186) was detected. Statistical analysis using one-way ANOVA showed significant difference between the three groups as shown in the (Figure 1).

Further, Bonferroni test showed significant difference (P < 0.001) among all three groups in regards to MMP-8 levels (Table 2).

**Correlation between salivary MMPs and clinical periodontal parameters**

No significant correlations were detected between the levels of the salivary MMP-8 and the clinical periodontal parameters.

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**Table 1: Comparison of the clinical periodontal parameters among the study groups.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (N=29)</th>
<th>Non-smokers (CP) (N=26)</th>
<th>Smoker (CP) (N=26)</th>
<th>Mean</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td></td>
<td></td>
<td></td>
<td>1.15</td>
<td>0.35</td>
<td>0.001**</td>
</tr>
<tr>
<td>GI</td>
<td></td>
<td></td>
<td></td>
<td>1.41</td>
<td>0.50</td>
<td>0.059</td>
</tr>
<tr>
<td>PPD (mm)</td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
<td>0.00</td>
<td>0.023*</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td></td>
<td></td>
<td></td>
<td>3.32</td>
<td>1.53</td>
<td>0.001**</td>
</tr>
</tbody>
</table>

Data are expressed as mean and standard deviation.

Group Non-smokers (CP) (non-smokers with chronic periodontitis), group smoker (CP) (smokers with chronic periodontitis).

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

*, ** significant
*** Highly significant
Figure 1: Salivary MMP-8 levels of three groups: Control, Group cpn: nonsmoking patients with chronic periodontitis, Group cps: smoking patients with chronic periodontitis. In smokers with chronic periodontist group, the levels of MMP-8 (ng/ml) were significantly increased as compared to group nonsmokers with chronic periodontitis and periodontally healthy groups (P < 0.001).

Table 2: Bonferroni test revealed significant difference among all three groups with respect to MMP-8 levels.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Non-smoker with chronic periodontitis</th>
<th>Smoker with chronic periodontitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.645</td>
<td>0.001**</td>
</tr>
<tr>
<td>Non-smoker with chronic periodontitis</td>
<td>0.645</td>
<td></td>
<td>0.006**</td>
</tr>
<tr>
<td>Smoker with chronic periodontitis</td>
<td>0.001**</td>
<td>0.006**</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Correlation between the level of MMP-8 and clinical periodontal parameter among non-smokers with chronic periodontitis group.

<table>
<thead>
<tr>
<th></th>
<th>Pearson Correlation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>0.244</td>
<td>0.230</td>
</tr>
<tr>
<td>GI</td>
<td>0.144</td>
<td>0.483</td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>0.299</td>
<td>0.137</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>-0.290</td>
<td>0.150</td>
</tr>
</tbody>
</table>

Table 4: Correlation between the level of MMP-8 (ng/ml) and clinical periodontal parameter among smokers with chronic periodontitis group.

<table>
<thead>
<tr>
<th></th>
<th>Pearson Correlation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>0.326</td>
<td>0.104</td>
</tr>
<tr>
<td>GI</td>
<td>0.051</td>
<td>0.806</td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>0.059</td>
<td>0.775</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>0.022</td>
<td>0.916</td>
</tr>
</tbody>
</table>

among the chronic periodontitis groups (smokers and non-smokers) (Tables 3,4).

**DISCUSSION**

The putative periodontal pathogens that are found in dental plaque can exert their potential virulence factors and thus they induce the host cells such as PMNs, gingival fibroblasts and epithelial cells to secrete their MMPs [27].

MMP-8 is currently regarded among the key biomarkers of inflammation, previous studies revealed that, salivary MMP-8 levels are higher in periodontitis patients, these findings reflect unique ability of MMP-8 to degrade collagen and hence explain its crucial role in periodontal diseases activity [28-30].

The quantitative analysis of salivary MMP-8 that performed in this study, demonstrated a clearly elevated level of salivary MMP-8 among smokers as compared with non-smokers and periodontally healthy subjects.

This can be explained by the detrimental effect of smoking in the periodontal tissues through interfering with vascular and inflammatory response together with its harming effect in the granulocytes function which acts as a first line of defense against microbial challenges by secreting high amount of inflammatory...
mediators one of which is the MMP-8. Thus, tobacco could influence MMP-8 levels in periodontal tissues of smokers [31].

Further this result highlighted the importance of doxycycline, a known inhibitor of PMN MMP-8 as a possible adjunctive drug in the treatment of chronic periodontitis [32].

Moreover, the present findings of significantly increased serum concentrations of MMP-8 among smoker chronic periodontitis patients deserve further investigation and suggest that chronic periodontitis together with smoking can predispose the development of cardiovascular diseases [33].

The results of the present study are in line with findings of other studies which revealed an increase in salivary MMP-8 level in smokers with chronic periodontitis as compared to non-smokers [34,35].

In contrast, a study conducted by Liede et al., suggested that smoking may significantly lower MMP-8 level in saliva. A fact well known, periodontal tissue breakdown is higher in smokers; it is therefore 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 [31,36,37].

According to this study no significant difference was found in gingival index (GI) between smokers, non-smokers’ chronic periodontitis groups and the periodontally health group, the results revealed that the chronic periodontitis group including both smokers and non-smokers patients associated with higher GI than periodontally healthy group.

This finding was in agreement with Nassrawin, and in disagreement with Calsina et al., [38,39].

Usually, reduced bleeding in smokers has been ascribed to gingival vasoconstriction caused by the actions of nicotine-stimulated adrenaline; nevertheless, the available evidence that supports this hypothesis in humans is not definite as smoking can cause vasodilatation in some tissues due to the action of noradrenaline on Al-adrenergic receptors [37].

The current study found that the value of pocket depth and clinical attachment loss was the highest among smokers as compared to nonsmokers and periodontally healthy groups, which is in agreement with the findings by Linden and Mullally. Furthermore Haffajee and Socransky got the same conclusion in regards to pocket depth in nonsmokers. In addition, the result of this was in agreement with the results by Grossi et al., who found strong association between smoking and attachment loss [40-42].

The greater amount of attachment loss and the highest pocket depth can be due to the a significantly higher levels of IL-1, IL-6, and TNF-α exhibited in smokers, which in turn stimulate the expression of the receptor activator of nuclear factor- kappa ligand (RANKL) and the inhibitor protein osteoprotegerin (OPG), which are crucial factors for bone resorption and remodeling [43].

The OPG concentration was significantly lower and the sRANKL/OPG ratio was higher in smokers than in non-smokers, in saliva along with serum, elucidating the greater potential for bone loss in smokers [44].

Moreover, in the present study no significant correlations were found between the clinical periodontal parameters and the level of salivary MMP-8 level among the smokers with chronic periodontitis and non-smoker chronic periodontitis groups. This could be due the size of the study sample, for instance most of linear correlations need a large sample size in order to detect the effect of the risk factors on the periodontal parameters.

This finding may support the hypothesis that salivary MMP-8 levels might reflect early development of periodontitis, therefore MMP-8 seems to be a key biomarker during the early stages of periodontal disease and other biomarkers may play more important role in the periodontal disease progression [45].

However, the findings of this study could be applied in most instances and in the light of the prevailing evidences which demonstrate the increase in salivary MMP-8 level among smokers with chronic periodontitis may contribute to increase in the periodontal attachment loss therefore MMP-8 may be a useful tool for monitoring periodontal disease activity among smokers.

**CONCLUSION**

The smokers with chronic periodontitis have the highest values of clinical periodontal parameters and this is reflected by the elevated level of Salivary MMP-8. However, no significant correlations were detected when a comparison was made between salivary MMP-8 and clinical periodontal parameters per group and this may be explained by the fact that our present material was a cross-sectional, and future longitudinal studies are needed for definite conclusions.

**LIMITATIONS OF THE STUDY**

Some limitations need to be addressed when extrapolating our current results for further research. First, this is a cross-sectional study without longitudinal observation, hence a more thorough investigation with a longitudinal design is still required to shed more light on the association of MMP-8 with the progression of periodontitis. A lack of detailed information about the number of cigarettes per day and duration of smoking was also a limitation of this study.

Moreover, the lack of females among the smoker’s subjects can weaken the results of this study however a possible justification for this limitation can be due to the low levels of tobacco consumed by women compared to men, attributable mainly to social pressures in the community that consider smoking by women as a major sin.

Therefore, the current results could only partly confirm our study hypothesis, necessitating the conduction of further studies with larger sample size and more comprehensive study design, thus yielding a better explanation of the intrinsic mechanism of the function of MMP-8 in periodontitis.

**ACKNOWLEDGMENT**

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REFERENCES


