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SHORT PAPER

Importance of MMP-8 in Salivary and Gingival Crevicular Fluids of Periodontitis Patients

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ABSTRACT

Background: Matrix metalloproteinases (MMPs) stimulate alveolar bone loss in chronic periodontitis. **Objective:** To evaluate the salivary and gingival crevicular fluid (GCF) levels of MMP-8 in patients with moderate to severe chronic periodontitis. **Methods:** 42 participants were divided into two groups: a case group (21 patients with generalized moderate to severe chronic periodontitis) and a control group (21 healthy periodontal subjects). GCF and saliva samples were obtained from both groups. Salivary and GCF MMP-8 levels of each subject were detected using the ELISA method. **Results:** Mean±SD values of salivary MMP-8 levels of the control and case groups were 1.52 ± 0.65 ng/ml and 6.06 ± 1.18 ng/ml, respectively, and statistically significant difference was observed (p=0.0001). Also, mean±SD values of GCF MMP-8 levels of the control and case groups were 0.87 ± 0.26 ng/ml and 2.92 ± 0.64 ng/ml, respectively; which was statistically significant (p=0.0001). **Conclusion:** Our results demonstrate an increased concentration of salivary and GCF levels of MMP-8 in the patient group.

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Keywords: Chronic Periodontitis, Crevicular Fluid, Gingiva, Matrix Metalloproteinase-8, Saliva

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INTRODUCTION

Chronic periodontitis is an inflammatory disease inducing extensive destruction of alveolar bone and periodontal ligaments (1). Disease progression and induced inflammation involves a network of mutual molecular pathways consisting of pro-inflammatory mediators such as cytokines, reactive oxygen species (ROS), matrix metalloproteinases (MMP), and their inhibitors and modulators (2-3). MMPs form a big family engaged in tissue remodeling and extracellular matrix degeneration (4). Pathogens in microbial plaque can stimulate host cells to increase MMP release. Any imbalance between MMPs and their inhibitors may stimulate degeneration of extracellular matrix (ECM) and alveolar bone (5-6). The most common MMPs associated with tissue degeneration belong to collagenase family and mainly include MMP-8 and MMP-13 with significant participation of MMP-9 and MMP-14 (7-8). MMP-8 has a unique ability in degeneration of collagen type I and III (9). Recent studies suggest that MMP-8 has a strong correlation with bleeding on probing, attachment loss, and probing pocket depth (10-11). Increased MMP-8 level in gingival crevicular fluid (GCF), saliva and infected tissue in chronic periodontitis patients reveal an important role of this biomarker in chair- side diagnostic tests of periodontitis (8). Recently, monoclonal antibodies are developed as diagnostic tests helping rapid detection of MMP-8 and thus differentiation of healthy areas from areas with gingivitis and periodontitis (10). Biomarkers are found in different body fluids such as serum, GCF, saliva, urine, etc. (12). Many efforts have been done over years to find a way to determine the prognosis of oral diseases by saliva analysis (13). Gupta *et al.* (2015) evaluated MMP-8 level in saliva in patients with chronic periodontitis in comparison with healthy individuals using ELISA method. They concluded that MMP-8 level in saliva of patients with periodontitis was higher compared with healthy individuals (7). Sorsa *et al.* (2020) demonstrated that active MMP-8 levels in mouth rinse of healthy subjects were significantly lower in comparison with patients with severe periodontitis stages and grades. The effects of aMMP-8 on oral health were found more detrimental than traditional periodontal parameters such as bleeding on probing (14). In a systematic review by Morais *et al.* (2017), high levels of MMP-8 were correlated with the development of gingivits to periodontitis (15). Rangbulla *et al.* (2018) evaluated salvia level of IgA, Interleukin-1B, and MMP-8 in patients with moderate to severe chronic periodontitis using ELISA method. They showed that saliva level of these biomarkers significantly declined in patients with moderate to severe chronic periodontitis 12 weeks after oral prophylaxis (16). Crudden *et al.* (2017) assessed MMP-8 activity in GCF in patients with periodontal diseases and concluded that MMP-8 activity can be specifically traced and quantified since measurement of MMP-8 activity is helpful in monitoring the progress of periodontal diseases (17). The present study intended to determine the level of MMP-8 in GCF and saliva of patients with generalized moderate to severe chronic periodontitis and healthy individuals.

MATERIALS AND METHODS

Patients and Ethics. Study population included patients with moderate to severe chronic periodontitis and healthy individuals referring to periodontology ward of dentistry faculty and Jahad Daneshghahi Center of Mashhad University of Medical

Sciences during 2017-2018. Participants were divided into two groups according to inclusion criteria and sample size. The case group consisted of 21 patients with generalized moderate to severe chronic periodontitis, and the control group consisted of 21 healthy individuals while trying to choose similar participants in terms of age and gender (the sample size was 42). The inclusion criteria consisted of the following:

1. Negative habitual history of smoking (both case and control groups)
2. Absence of systemic inflammation, diabetes, autoimmune diseases, and immunodeficiency disorder (both case and control groups)
3. No history of antibiotic, NSAID, or corticosteroid consumption in the last 6 months (both case and control groups)
4. Absence of periodontal disease (control group)

In this study, 21 patients with generalized moderate to severe chronic periodontitis in the case group and 21 healthy individuals in the control group were evaluated. Case group consisted of 11 female participants (52.38%) and 10 male participants (47.61%), while the control group consisted of 10 female participants (47.61%) and 11 male participants (52.38%). Mean age of participants in the case group was 42.4 ± 6.9 ranging from 30 to 56, while mean age of control group was 30.1 ± 3.6 ranging from 25 to 37 (Table 1).

Table 1. Demographic data of participants in case and control groups.

Feature	Control group N=21	Case group N=21
Age	30.1±3.6	42.4±6.9
Gender (Male)	52.38%	47.61%
Gender (Female)	47.61%	52.38%

Before sampling, informed consent was obtained from participants. The study was approved by organizational ethics committee of faculty/regional of Mashhad University of Medical Science with code number 960238 and approved with IR.mums.sd.REC.1394.267. Gingival index (GI) and plaque index (PI) were measured according to Silness and Loe study (18-19) and probing depth (PD) (the distance between gingival margin and depth of gingival sulcus) and clinical attachment level (CAL) (the distance between cementoenamel junction (CEJ) of teeth and the depth of gingival sulcus) by Williams probe were measured in four areas around each tooth. To prevent measurement bias, all indexes were measured by the same examiner.

Sampling of gingival crevicular fluid (GCF) and saliva. For GCF collection, paper cone number 40 was mildly put into gingival sulcus. After 30 seconds, the cone was removed and put into micro tubes containing 0.2 ml of phosphate-buffered saline (PBS). In patients with moderate to severe chronic periodontitis, sampling was performed from the tooth with the highest level of attachment loss and inflammation signs, and in healthy individuals, samples were obtained from normal sulcus of posterior teeth, left and lower quadrant. Saliva was collected according to Navazesh protocol 1993 (19). The participants were banned from teeth brushing in the last 12 hours and eating and drinking in the last 1 hour before saliva sampling (except water drinking). Saliva sampling was performed without salivation stimulation. Before saliva sampling, participants were asked to wash their mouth with 150 ml water and sit relaxed for 5

minutes having no body movement, especially mouth movement, so approximately 5 ml of saliva was gathered in clean disposable cups. Saliva samples were transferred by syringe to codified micro tubes. Then samples were frozen at -20ºC in the immunology lab of Mashhad University of Medical Sciences.

ELISA. This test was conducted according to ELISA kit (Human Matrix Metalloproteinase 8/Neutrophil Collagenase (MMP-8) Elisa kit/ Bioassay Technology Laboratory). 50 µl of standard concentration, 40 µl of saliva and GCF sample, and 10 µl of anti-MMP-8 antibody were added, and then 50 µl of Streptavivin-HRP was added. The plate was properly mixed, covered with sealer, and incubated at 37ºC for 60 minutes. Washing took place five times. Afterwards, 50 µl of substrate solution A and then 50 µl of substrate solution B were added. The plates were covered with new sealers and incubated at 37ºC for 10 minutes in the dark. 50 µl of stop solution was added to stop the reaction, and rapid color change from blue to yellow was observed in this step. In the last step, optical density of each well was determined 10 minutes after adding stop solution at 450 nm. Finally, MMP-8 concentration of samples was calculated using standard chart.

Statistical Analysis. Herein, the clinical parameters are quantitative variables; therefore, independent t-test was used for comparison and Pearson test was applied for data analysis of MMP-8 level in the study groups.

RESULTS AND DISCUSSION

Mean and standard deviation of studied clinical parameters (PD-CAL-GI-PI) in the case and control groups are presented in Table 2. Mean and standard deviation of saliva MMP-8 level in the case group was 6.06 ± 1.18 ng/ml ranging from a minimum of 4 ng/ml to a maximum of 8 ng/ml with a median of 5.9 ng/ml. In the control group, it was 1.52 ± 0.65 ng/ml ranging from a minimum of 0.6 ng/ml to a maximum of 2.8 ng/ml with a median of 1.3 ng/ml. These values in the case and control groups presented a statistically significant difference (p=0.0001). Figure 1 compares saliva MMP-8 level in the case and control groups.

Table 2. Clinical parameters in case and control groups (mean ±SD)

	PD	CAL	GI	PI
Control	2.61±0.49	0	0	**1.09±0.3**
Case	5.90±0.88	4.61±1.32	2.28±0.56	**1.9±0.53**
p value	p=0.0001	p=0.0001	p=0.0001	**p=0.0001**

PD: probing depth; CAL: clinical attachment level; GI: gingival index; PI: plaque index.
Independent T-test was used to compare the two groups.

The mean and standard deviation of GCF MMP-8 level in the case group was 2.92 ± 0.64 ng/ml ranging from a minimum of 1.3 to a maximum of 4.1 ng/ml with a median of 2.9 ng/ml, while it was 0.87 ± 0.26 ng/ml ranging from a minimum of 0.4 to a maximum of 1.5 with a median of 0.9 ng/ml in the control group.

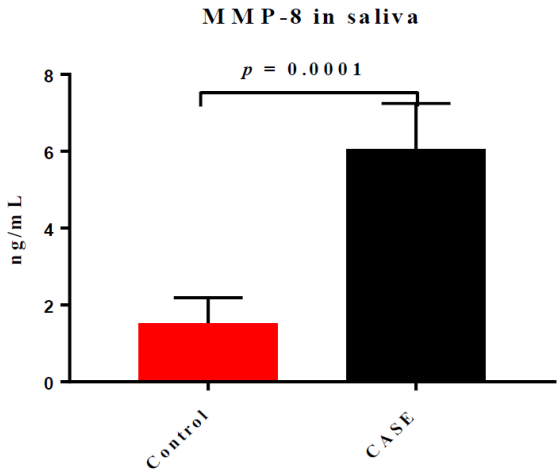

Figure 1. Comparison of saliva MMP-8 level in case and control groups.

These values presented a statistically significant difference (p=0.0001). Figure 2 compares GCF MMP-8 level in the case and control groups. Comparing mean increase in MMP-8 level in saliva and GCF in the case group with the control group, it is observed that the mean MMP-8 level in saliva in the case group is four times more than the MMP-8 level in saliva of the control group.

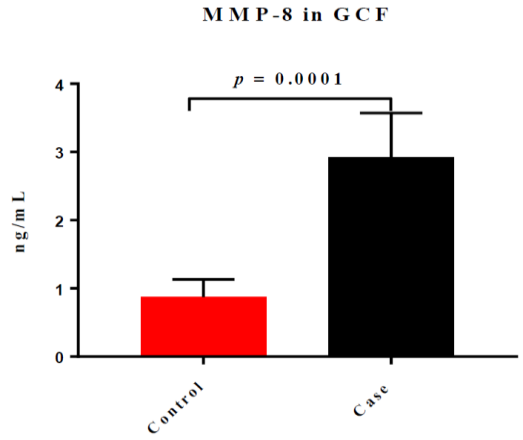

Figure 2 Comparison of GCF MMP-8 level in case and control groups.

Moreover, mean MMP-8 level in GCF in the case group is 3.6 times more than the control group. Furthermore, MMP-8 level in saliva and GCF was higher in all patients in the case group compared with patients in the control group. Figure 3 demonstrates the comparison of increased level of MMP-8 in saliva and GCF of the case and control groups. Table 3 summarizes the evaluation results of the correlation between periodontal parameters and MMP-8 level in saliva and GCF. The most prominent

finding of this study is the significant difference in MMP-8 level in saliva and GCF between patients with generalized moderate to severe chronic periodontitis and healthy individuals.

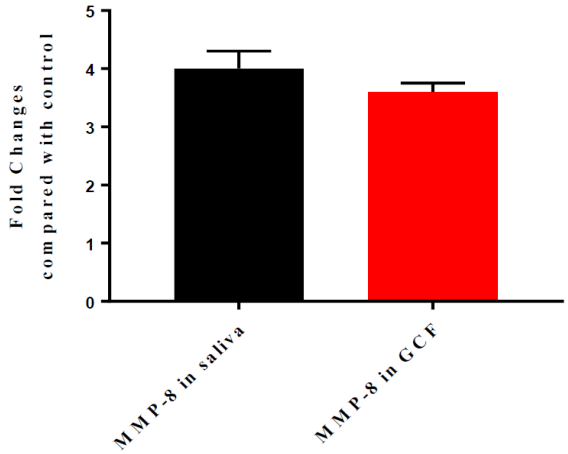

Figure 3 Comparative chart of increase level of MMP-8 in GCF and saliva.

The results demonstrated a significant correlation between the studied clinical parameters (PD-CAL-GI-PI) and MMP-8 level in saliva and GCF. Meanwhile, the weakest correlation was observed between PI and GCF level of MMP-8, and the strongest correlation was found between CAL and GCF level of MMP-8. Expression of MMP-8 in GCF of severe chronic periodontitis was significantly associated with CAL, which is a clinical parameter of periodontitis severity.

Table 3. Results of evaluation of correlation between periodontal parameters with MMP-8 level.

	PD	**CAL**	**GI**	**PI**
MMP-8 level in saliva	r=0.82 95% CI; 0.69-0.90 *p= 0.0001*	r=0.88 95% CI; 0.79-0.93 *p= 0.0001*	r=0.88 95% CI; 0.79-0.93 *p= 0.0001*	r=0.68 95% CI; 0.48-0.82 *p= 0.0001*
MMP-8 level in GCF	r=0.83 95% CI; 0.71-0.91 *p= 0.0001*	r=0.92 95% CI; 0.85-0.95 *p= 0.0001*	r=0.85 95% CI; 0.74-0.91 *p= 0.0001*	r=0.49 95% CI; 0.22-0.69 *p= 0.0008*

MMP-8 has been suggested to be a central mediator in chronic infection and implicated in the progression of periodontitis by regulating collagen degradation (20). It can be concluded that the amount of dental plaque is more related to hygiene status of patients at sampling with no such relationship with MMP-8 level. Additionally, the strong correlation between CAL and GCF level of MMP-8 can indicate that chair-side test may be designed for measurement of MMP-8 level in GCF to determine individuals' periodontal status. Mantyla *et al.* (2003) demonstrated chair-side monitoring of MMP-8 in GCF of periodontal patients with a test stick. They found out that the MMP-8 test can

be an important way to define periodontitis from gingivitis and healthy subjects. If MMP-8 level of GCF was higher than 1 mg/l and chair-side test of MMP-8 was positive, implied that the patient has severe periodontitis. The MMP-8 levels in subjects with positive MMP-8 test and probing pocket depth more than 5 mm (before treatment) can be reduced by scaling and root planning (21). On the other hand, there is a strong correlation between CAL and saliva MMP-8 level. Accordingly, saliva level of MMP-8 is a suitable alternative for MMP-8 level in GCF. In a systematic review and meta-analysis by Lin Zhang and *et al.* (2018), the level of this salivary biomarker was considered for early diagnosis of periodontitis (22). Rai *et al.* assessed the level of MMP-2 and MMP-9 in GCF and MMP-8 in the saliva of patients with gingivitis and periodontitis in comparison with healthy individuals by ELISA (9). They demonstrated that the level of MMP-8 in saliva and MMP-9 in GCF was significantly higher in patients with periodontitis compared with healthy individuals. In other words, MMP-2 level in GCF of patients with periodontitis is lower compared with patients with gingivitis and healthy individuals. The level of these three MMPs showed a strong relationship with clinical parameters such as probing depth and bleeding on probing. In contradiction, the present study performed simultaneous assessment of MMP-8 level in saliva and GCF. Also, the correlation between MMP-8 level and CAL parameter in the study of Rai et al. was reported to be r=0.52, while in our study, r=0.88. Gupta *et al.* assessed the level of MMP-8 in saliva of chronic periodontitis and compared it with healthy individuals using ELISA (7). They stated that the level of MMP-8 in the saliva of patients with chronic periodontitis was higher compared with healthy individuals. They reported that MMP-8 level in saliva of patients with chronic periodontitis was 348.76 ± 202.1 ng/ml and 190.91 ± 143.89 ng/ml in control group. In the current study, MMP-8 level in saliva was reported to be 6.06 ± 1.18 ng/ml for patients with chronic periodontitis and 1.52 ±0.65 ng/ml, which can be attributed to ethnicity and race differences. Rangbulla *et al.* evaluated and compared IgA, Interlukin-1β, and MMP-8 level in saliva of patients with moderate to severe periodontitis and healthy individuals using ELISA (16). They demonstrated that saliva level of MMP-8 in patients with moderate to severe chronic periodontitis significantly decreased 12 weeks after oral prophylaxis.

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