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Original article

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Matrix Metalloproteinase-3 but Not Matrix Metalloproteinase-9, Implicated in the Manifestation of Chronic Periodontitis

Pouyan Razavi[1], Seyed Abdolrahim Rezaee[2], Salehe Akhondian[3], Nastaran Asgari[3], Kazem Fatemi*[4], Farnaz Mohajertehran*[4]

Abstract

Background: Periodontal disease is an inflammatory condition affecting the tooth's supporting tissues, resulting in gradual loss of periodontal ligament (PDL), alveolar bone, and gum resorption. Neutrophil and monocyte/macrophage, destructive proteases like matrix metalloproteinase (MMP)-3 and MMP-9 play pivotal roles in such lesions in periodontitis. Therefore, this study aims to compare the level of MMP-3 and MMP-9 gene expression in patients with or without periodontitis in an Iranian population.

Methods: This cross-sectional study was carried out on 22 chronic periodontitis patients and 17 healthy control subjects referred to the department of periodontology, Mashhad Dental School. In both groups, the gingival tissue was removed during surgery and transferred to the Molecular Biology Laboratory for MMP-3 and MMP-9 gene expression evaluation. The qRT-PCR, TaqMan method was used for gene expression assessments.

Results: The average age of periodontitis patients was 33± 5 years, and in controls, 34.7 ±6 with no significant differences. The mean MMP-3 expression in periodontitis patients was 146.67±38.7, and in controls, 63.4±9.1. The difference was statistically significant (P=0.04). The mean expression of MMP-9 in periodontitis patients and controls were 103.8± 21.66 and 87.57± 16.05, respectively. Although the target gene expression in patients was higher, the difference was insignificant. Furthermore, there was not any significant correlation between age or gender with the expression of MMP3 or MMP9.

Conclusions: The study demonstrated that the MMP3 seems to have a destructive impact on the gingival tissue in chronic periodontitis, but not MMP9.

Keywords: Chronic periodontitis, Matrix metalloproteinase-3, Matrix metalloproteinase-9.

Introduction

Periodontitis is a complex inflammatory condition of periodontal tissues consisting of the gingiva, cementum, alveolar bone, and periodontal ligament, which, if uncontrolled, can result in tooth loss and bone destruction. Chronic periodontitis and aggressive periodontitis are the two main varieties, and it is one of the most prevalent disorders in the world, with a frequency of 15–20% (1).

Bacterial dental plaque is the leading cause of periodontal diseases, which comprises a mixture of bacteria in diverse combinations of different pathogenic potency (2, 3). In addition to bacteria-induced destruction, periodontal degradation is aided by host immune-inflammatory responses caused by bacterial antigens and lipopolysaccharides (LPS) (4). Furthermore, the disease

1: Student Research Committee, Faculty of Dentistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.
2: Immunology Research Center, Inflammation and Inflammatory Diseases Division, Mashhad University of Medical Sciences, Mashhad, Iran.
3: Student Research Committee, Faculty of Dentistry, Mashhad University of Medical Science, Mashhad, Iran.
4: Oral and Maxillofacial Disease Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
***Corresponding author: Kazem Fatemi; Tel: +98 51 38832300; E-mail: FatemiK@mums.ac.ir**
& Farnaz Mohajertehran; Tel: +98 51 38832300; Email: mohajertf@mums.ac.ir.
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development is influenced by various pro-inflammatory mediators such as cytokines, reactive oxygen species, and matrix metalloproteinase (5, 6).

Matrix metalloproteinases (MMPs) are metal-dependent proteases that break down extracellular matrix and basement membranes (7, 8). MMPs are the most critical proteinases for the deterioration of matrix proteins in periodontitis. Any mismatch between MMPs and their inhibitors will result in ECM, basement membrane, and alveolar bone destruction (9). In addition, pathogens in dental plaque may cause host cells to produce more MMPs, which leads to more tissue loss in periodontitis (10) (Fig. 1).

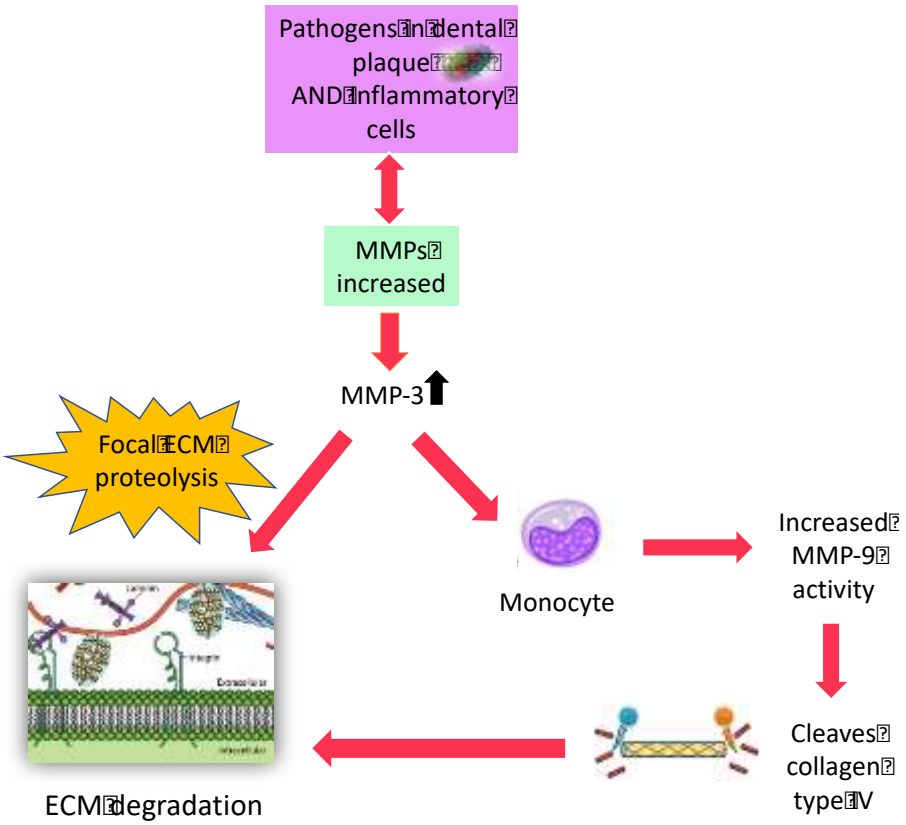

Fig. 1. Molecular mechanisms of MMP-3 and MMP-9 leads to Extracellular matrix degradation.

Matrix metalloproteinases are divided into six categories based on substrate specificity, sequence similarity, and domain organization: collagenases, stromelysins, matrilysins, membrane-type MMPs, and other MMPs (11). The collagenases family of MMPs is the most prevalent form of MMPs linked to tissue degradation, with MMP-8 and MMP-13 playing a significant role. MMP-9 and MMP-14 also play an essential role (12). Stromelysin-1 (MMP-3) has been related to tissue degradation in chronic inflammatory diseases like periodontitis due to its considerable substrate specificity and can play a crucial role in triggering latent pro-MMP-1 pro-MMP-8 pro-MMP-9 (13). MMP-9 is a proteolytic enzyme regulated by cytokines and secreted by active monocytes and lymphocytes that cleaves denatured collagen, especially collagen type IV, the basement membrane's main component (14).

While several studies have assessed altered MMP gene expression levels in periodontitis patients of various races and ethnicities throughout the world (15, 16), no research has been discovered to investigate this in an Iranian population. Therefore, this study aims to identify a link between MMP-3 and MMP-9 gene expression and chronic periodontitis in an Iranian population, based on the role of MMPs, particularly MMP-3 and MMP-9, in the pathologic process of periodontitis.

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Materials and Methods

In this cross-sectional study, 42 patients from the department of periodontics, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran, were enrolled in this study through convenience sampling. The included population consisted of two groups based on clinical periodontal condition, which were as follows: (1) Test group patients with generalized moderate/severe chronic periodontitis (n=20) and (2) Control group patients with a healthy periodontium (n=22). Patients with a systemic disease or infection, smoking habit, or a history of periodontal treatment, antibiotics, or NSAIDs consumption in the past six months were excluded from this study. Patients were labeled healthy if they met the following criteria: maximum probing depth of 3mm, no periodontal pocket, and no clinical attachment loss. In the periodontitis group, patients with 4mm or more clinical attachment loss, probing depth of at least 5mm, bleeding on probing, and involvement of 30% of the teeth or more were selected based on the American Academy of Periodontology standards.

In compliance with the declaration of Helsinki, after a thorough explanation of the method of the study, all patients signed written informed consent. The Ethics Committee approved the study of Mashhad University of Medical Sciences under the reference code of IR. MUMS.1397.322.

Sampling and RNA extraction

Each study participant had a small gingival biopsy taken after recording the periodontal parameters. Gingival samples from the test group were taken during surgical debridement after initial therapy from sites with a probing depth of 5mm or more. In contrast, samples from the healthy group were taken during an esthetic crown lengthening procedure or a simple erupted third molar extraction from periodontal healthy sites with a maximum probing depth of 3mm and no bleeding during probing. Each gingival sample was made of connective tissue and epithelium and was cut using a surgical curette and immediately suspended in a stabilizing buffer (RNA later, Qiagen, Germany) at –20 °C till collection of all eligible samples. Genomic RNA extractions were done by any tissue homogenization using an autoclaved mortar and pestle in the presence of RiboPure Lysis Buffer (RLT). The mixture was centrifuged for 4 minutes at 14800 RPM supernatant was transferred to a tube containing 70% ethanol. RNeasy mini kit (Qiagen, Germany) was used to collect total RNA from samples based on the manufacturer's instructions. Soon after that, cDNA synthesis was applied to all samples.

cDNA synthesis and real-time PCR

After extraction of total RNA, a RevertAid First Strand cDNA Synthesis Kit (Thermofisher Scientific, USA) was used to synthesize complementary DNA by reverse transcription of total RNA based on the manufacturer's instructions. PCR with GAPDH-specific primers was used as an internal control to approximate the effectiveness of cDNA synthesis from total RNA.

The qRT-PCR, TaqMan method was applied using a Q 6000 machine (Qiagen, Germany) by the designed primers and probes. Table 1 shows the primers and probes oligonucleotides. The two standard curves, relative gene expression analysis, were carried out on genomic cDNA for MMP-3 and MMP-9, and beta-2 microglobulin (β2M) as a reference gene as previously described (17).

After preparing the standard curves for the target and reference genes and analyzing the data by Rotor-gene 6000 software (Qiagen, Germany), the expression index was calculated by normalizing the relative quantity of the interested genes to the relative quantity of β2M as the reference gene. Normalized Index = copy number of the gene of interest (MMP-3, MMP-9) / copy number of reference gene (β2M) (Fig. 2).

Statistical analysis

The data were analyzed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA). The variations in MMP-3 and MMP-9 expression levels between the control and test groups were analyzed using the Mann-Whitney test after the normality of the data was evaluated using the Kolmogorov-Smirnov test. In order to compare the differences in mean age and gender

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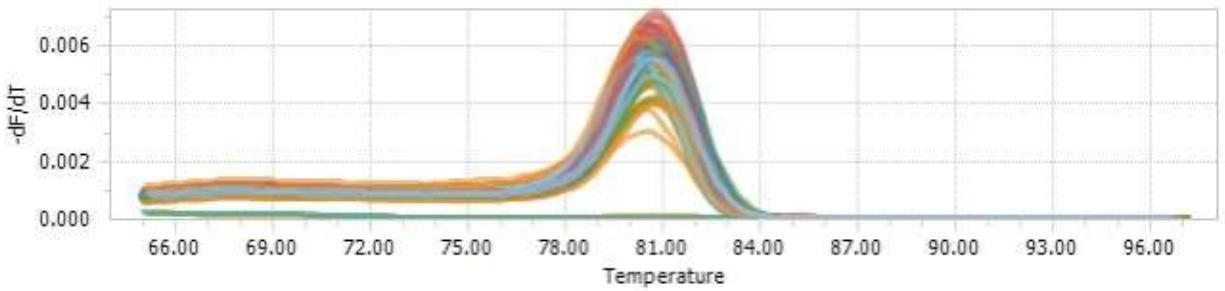
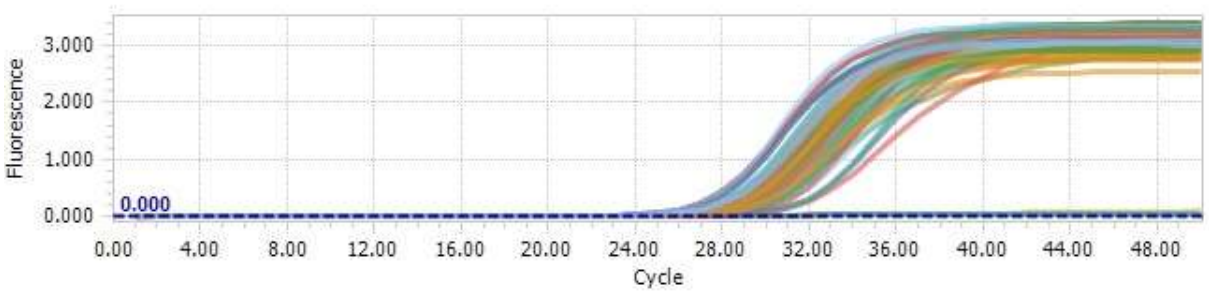
distribution among the two groups, an independent T-test and Fisher exact test were used, respectively. In addition, the association between MMP-3 and MMP-9 expression levels in both groups was determined using a Pearson's correlation coefficient test.

Table 1. Sequence of primers.

Gene		Sequence (5'→3')	Length (bp)	Product size (bp)
MMP-3	Forward	CCCACTCTATCACTCACTCACAG	23	179
	Reverse	CAAAGGACAAAGCAGGATCACAG	23	
MMP-9	Forward	CAGCGAGAGACTCTACAC	18	154
	Reverse	GTCCCGGTCGTAGTTG	16	
Beta2 microglobulin	Forward	TTGTCTTTCAGCAAGGACTGG	21	127
	Reverse	CCACTTAACTATCTTGGGCTGTG	23	



a)



b)

Fig. 2. The melting curve (a) and amplification plot (b) of MMP-3 and MMP-9 expression in real time PCR.

Results

Patient's characteristics

Twenty healthy patients and 22 patients with chronic periodontitis were enrolled. However, three subjects from the healthy group were lost to drop out. The demographic characteristics and gene expressions of the remaining 39 participants are mentioned in Table 2. Even though females made up 80.9 percent of the people studied, there was no statistically

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significant difference in gender distribution between the groups. Furthermore, the mean age difference between the groups was not statistically significant.

Table 2. Demographic and genes expressions information of the study participants.

		Chronic Periodontitis group (n=22)	Healthy control group (n=17)	P-value
Gender	Female	18	13	1.000
	Male	4	4	
Age Average		33.6 ± 5	34.7± 5.3	0.132
MMP-3 gene		146.67± 38.74	63.4 ± 9.1	0.048
MMP-9 gene		103.8 ± 21.66	87.57 ± 16.05	0.951

Gene expression

According to the findings of this study, the mean gene expression of MMP-3 and MMP-9 was higher in the case group than in the control group. In this case, however, the median of the data was utilized to represent the data because it was not normally distributed, and there were many outliers. In general, in the MMP-3 group, one extreme outlier was removed from the case group and one from the control group. Although the mean expression level of MMP-9 was higher in the case group than in the control group, these differences were found to be not statistically significant. However, for MMP-3, the situation was different, so along with the increase in mean expression in the case group, this difference was significant. A minor relationship between MMP-3 and MMP-9 was found in both the case and control groups. However, this relationship was not statistically significant (case P=0.9030, control P=0.5410).

Discussion

The current study's findings show that participants with periodontitis had elevated mean MMP-3 and MMP-9 gene expression .In this regard, the difference in gene expression was significant in the MMP-3 group but not in the MMP-9 group. These results are scarcely distinguishable from Astolfi et al.((18), which found that the MMP-3 gene polymorphism may contribute to periodontal tissue destruction during periodontitis in Brazilian subjects MMP-3 is needed for demolishing ECM proteins such as laminin and the cascade activation of other MMPs, including MMP-1,-8, and -9 (19). These findings contrast with those of Kubota et al. (20), which found a statistically non-significant rise in MMPs gene expression in patients with periodontitis. Additionally, the Itagaki et al. (21) study, which did not show a significant difference for the MMP-3 in a Japanese population, was also contrary to the current study's findings. Hsiao et al. (22) reported a significant effect of the MMP-9 gene in a Taiwanese population MMP-9, which has been shown to have an action against collagen and proteoglycans and is found on chromosome 20q11.2-q13.1, is an essential mediator in periodontitis formation (23, 24). There is other conflicting evidence that the MMP-9 polymorphism may be a protective factor for Chronic Periodontitis, especially in Caucasians and Asians (25). The discrepancy might be related to genetic variations across races. In another study with a different method, due to the high diagnostic power of the saliva algorithm for the MMP-9 gene, the prognostic ability of this gene for periodontitis was reported positively (23).

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However, due to the limited number of samples, these findings should be interpreted cautiously. An additional source of error is that our samples were taken after phase 1 of periodontal therapies, consisting of scaling and root planning. This procedure might be a reason for the lower expression of MMP-3 and MMP-9. Therefore, further studies with bigger sample sizes and studies on untreated tissues are required to interpret these findings.

In conclusion, this study was designed to find an association between MMP-3 and MMP-9 gene expression and chronic periodontitis ,and in this regard, this association was observed to be significant for MMP-3 in contrast with MMP-9. Given the contradictions that existed in the results of the papers, our study provides the framework for future investigations on this matter. Thus, despite its limitations, the results of this study might be of assistance in monitoring periodontal health and devising a diagnostic strategy for chronic periodontitis.

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Conflicts of Interest

The authors have no conflict of interest to declare.

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