

Alliance of Matrix Metalloproteinase-9 Promoter-1562C/T Polymorphism and Metalloproteinase-9 Saliva Concentration of Iraqi Patients with Chronic Periodontitis

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Abstract

Matrix metalloproteinases (MMPs) are key enzymes responsible for matrix degradation, derived from polymorphonuclear leukocytes, during the early stages of periodontitis. There is plenty of evidence for the role of matrix metalloproteinases in the destructive processes of periodontal disease as a viable target in early diagnosis and chemotherapeutic approach. The aim of this study was to determine the levels of salivary matrix metalloproteinase-9 (MMP-9) in patients with periodontitis and healthy controls and the role of gene polymorphisms in the aetiology of the disease. Levels of MMP-9 in saliva was determined by enzyme linked immune sorbent assay (ELISA) technique in healthy subjects without any periodontal disease (n = 45) and in the patients with diagnosed periodontitis (n = 45). Gene Amplification, gel electrophoresis and genotyping were also carried out on MMP-9 gene. The MMP-9-1562C/T appeared in three genotypes: CC, CT and TT, allele specific gene polymorphism was amplified by PCR technique. The distribution of different genotypes in patients and controls for each of MMP-9-1562C/T polymorphism was in a good accordance with Hardy-Weinberg equilibrium (HWE). Genotypes in both dominant and recessive models were non-significantly associated with periodontitis. Significantly higher salivary MMP-9 was observed in cases of periodontitis compared to healthy adults (p = 0.043). Salivary MMP-9 may serve as a biomarker of periodontal disease monitoring and aid in the early detection of periodontitis.

Key words: Polymorphism, matrix metalloproteinase-9, chronic periodontitis, ELISA, saliva, promoter -1562 C/T.

Introduction

Chronic periodontitis (CP), the most commonly occurring and slowly progressive form of periodontal disease, can lead to continual inflammatory host response, which may finally result in periodontal

attachment loss and bone resorption ^(1,2). The CP is a highly prevalent disease and has shown to affect 90 % of the worldwide individuals ⁽³⁾. Even though the key etiological factor that results in progression of CP is the formation of complex biofilm on the surfaces of teeth adjacent to their periodontal tissues, determinants like demographic, social, environmental, behavioural, systemic and genetic factors have also been coupled with the epidemiology of this disease ⁽⁴⁾.

Matrix metalloproteinase-9 (MMP-9) is a member of a family of proteolytic enzymes that

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regulate cell matrix composition by requiring zinc for their proteolytic activities. The MMP-9 cleaves denatured collagen (gelatin), in particular, type IV collagen, which constitutes the major component of the basement membranes ^(5,6). This cleavage helps lymphocytes and other leukocytes like dendritic cells (DCs) to enter and leave the blood and lymph circulations. The MMP-9 also cleaves myelin compounds such as myelin basic protein (MBP) and type 2 gelatin, which leads to remnant epitopes that can generate autoimmunity ^(5,6,7). Expression and secretion of MMP-9 by activated lymphocytes and monocytes is tightly regulated by cytokines, chemokines, eicosanoids and peptidoglycans ⁽⁷⁾. In most cell types, gene transcription of MMP-9 is inducible by cytokines and cellular interactions. The MMP-9 is secreted as a zymogen (proenzyme), which remains inactive unless it is activated by the removal of the propeptide domain by proteolytic enzymes like stromelysin-1, MMP-2 and other MMPs ⁽⁶⁾.

The aim of this study was to determine the role of gene polymorphisms in matrix metalloproteinase-9

gene in chronic periodontitis and investigate the association of gene polymorphism in this gene using the saliva MMP-9 concentration.

Methods

This study included 45 patients with chronic periodontitis and 45 healthy controls. Blood and saliva samples were taken from all participants, DNA was extracted from whole blood for all participants, the Matrix metalloproteinase-9 (MMP-9) gene fragment corresponding the MMP-9-1562C/T SNP was amplified with specific primers using conventional PCR technique. Genotyping was achieved through restriction fragment length polymorphism (RFLP)-PCR. Enzyme linked immune sorbent assay (ELISA) technique was used to measure the saliva concentration of MMP-9.

Results

A specific pair of primary was used to amplify Matrix metalloproteinase-9 (MMP-9) gene fragment corresponding the MMP-9-1562C/T polymorphism using PCR, as shown in figure (1).

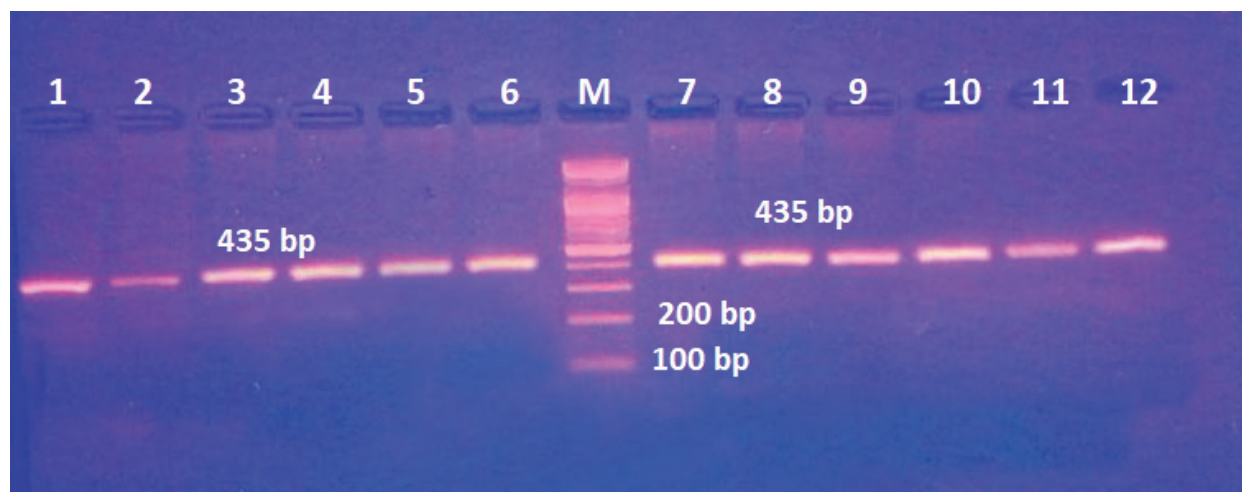


Figure (1): Gel electrophoresis of MMP-9-1562C/T gene polymorphism amplified with specific pair of primers using conventional PCR. The PCR product was stained with ethidium bromide. The fragment length was 435 base pair (bp).

Genotyping was achieved through RFLP-PCR. According to the cutting pattern, the MMP-9-1562C/T appeared in three genotypes: CC, CT and TT (Figure 2).

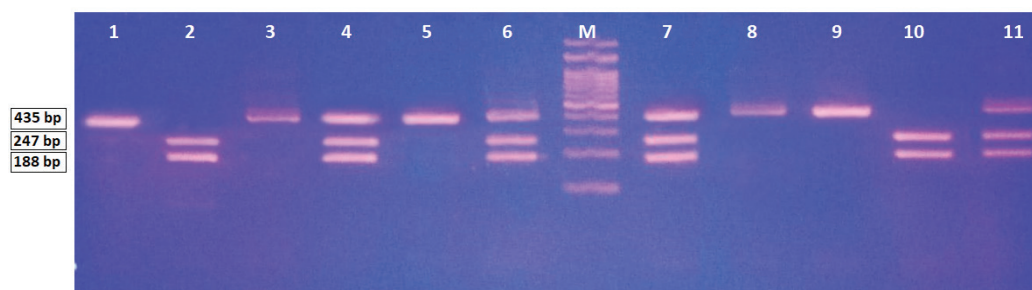


Figure (2): Sph I restriction endonuclease restriction fragment length polymorphism analysis of the MMP-9-1562C/T biallelic polymorphism, M, DNA ladder; lanes 1, 3, 5, 8 and 9: GG genotype; lanes 2 and 10: TT genotype; lanes 4, 6, 7, and 11: GT genotype.

bp: base pair.

Table (1) shows that the frequency of CC, CT and TT genotype in patient with periodontitis was 53.33 %, 40 % and 6.67 % respectively which did not differ significantly from that of the healthy controls (68.69 %, 28.89 % and 2.22 %, respectively). Moreover,

genotypes in both dominant and recessive models were non-significantly associated with periodontitis. Although the frequency of T allele was higher in the patients than that of the controls (26.67 % versus 16.67 %), the difference did not reach the significant level (OR= 1.82, 95 % CI= 0.88-3.75, p= 0.106).

Table (1): The frequency of different genotypes and alleles of MMP-9-1562C/T polymorphism in both patients and controls.

MMP-9-1562C/T Polymorphism	Controls (45)	Patients (45)	P-value (*)	OR (95% CI)
Genotypes				
CC	31 (68.69 %)	24 (53.33 %)	0.274 (NS)	1.0 Reference
CT	13 (28.89 %)	18 (40 %)	0.201 (NS)	1.8 (0.73-4.36)
TT	1 (2.22 %)	3 (6.67 %)	0.254 (NS)	3.87 (0.38-39.63)
HWE	0.788	0.879		
Dominant model				
CC+CT	44 (97.78 %)	42 (93.33 %)	0.33 (NS)	1.0 Reference
TT	1 (2.22 %)	3 (6.67 %)		3.14 (0.31-31.42)
Recessive model				
CC	31 (68.69 %)	24 (53.33 %)	0.132 (NS)	1.0 Reference
CT+TT	14 (31.11 %)	21 (46.67 %)		1.94 (0.82-4.58)
Alleles				
C	75 (83.33 %)	66 (73.33 %)	0.106 (NS)	1.0 Reference
T	15 (16.67 %)	24 (26.67 %)		1.82 (0.88-3.75)

(*) NS: Non significant at P value >0.05; C. I.: Confidence interval; OR: odds ratio; MMP-9: Matrix metalloproteinase-9; HWE: Hardy-Weinberg equilibrium.

The concentration of MMP-9 in saliva in the patient group (the mean \pm Standard deviation) was (10.27 ± 4.07) ng/mL while in the control group, it was (7.67 ± 3.35) ng/mL. There is a significant increase in patient in comparison to the control group ($p = 0.043$).

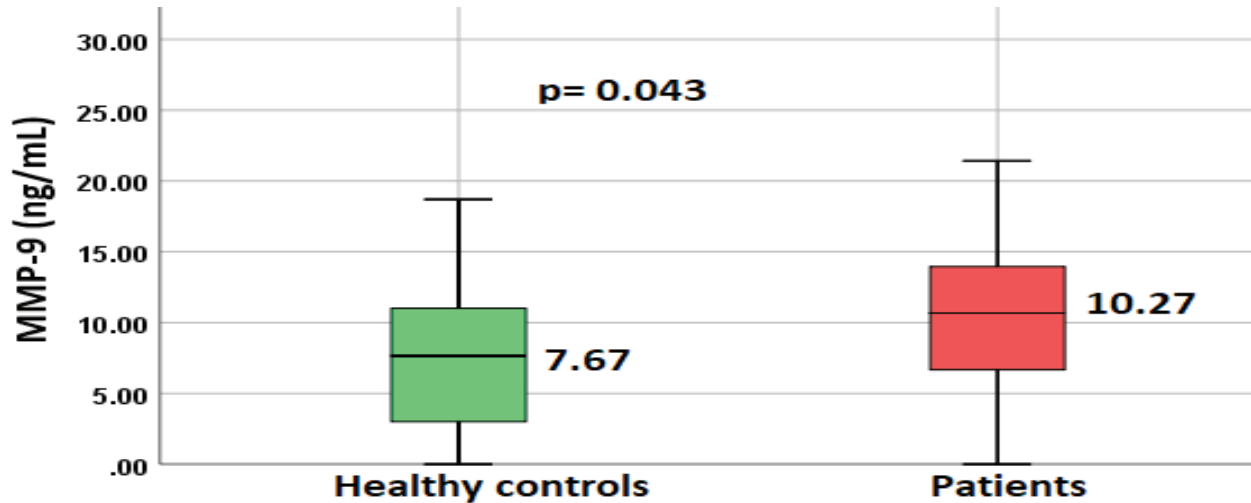


Figure (3): Mean concentration of Matrix metalloproteinase-9 (MMP-9) in saliva of both patients and controls. We note significant increase at P value <0.05.

Discussion

Our results show that there was no statistically significant difference in the frequency of the MMP-9-1562C/T genotypes between the periodontitis patient and the control persons, with no association between MMP-9-1562C/T polymorphism and the occurrence of periodontitis. Furthermore, genotypes in both dominant and recessive models were not significantly associated with periodontitis. The meta-analysis study of (Song et al., 2013) ⁽⁸⁾ demonstrates that a lack of association between the MMP-9-1562C/T polymorphisms and periodontitis. However, (Pan et al., 2013) study ⁽⁹⁾ found that the MMP-9-1562C/T polymorphism was associated with modified risk of periodontitis among Caucasian population, which population that differ from middle east population such as Iraq. While (Emingil et al., 2014) study ⁽¹⁰⁾ suggested that the other MMPs polymorphism such as MMP-8 might be associated with the susceptibility to periodontitis in Turkish population that also differ

from Iraq population.

In this present study, the salivary MMP-9 levels (mean \pm Standard deviation) in the periodontitis patient group was (10.27 ± 4.07) ng/mL while in the control group, it was (7.67 ± 3.35) ng/mL with a significant increase in patients comparison to the control group ($p = 0.043$). Our results were in agreement with many studies such as (Rai et al., 2008) ⁽¹¹⁾, (Wan et al., 2014) ⁽¹²⁾ and (Baeza et al., 2016) ⁽¹³⁾ studies. Other studies, such as (Wang et al., 2013) ⁽¹⁴⁾ and (Cheng et al., 2014) ⁽¹⁵⁾ studies, proved that the MMP-9 levels could also predict the prognosis and progression of many disorders in nasopharyngeal region including chronic inflammation and carcinoma, e.g. squamous cell carcinoma.

However, (Franco et al., 2017) meta-analysis study ⁽¹⁶⁾ established that the other genotype of MMP-9 such as MMP-9-753 C/T polymorphism reduced the risk of chronic periodontitis but not mentioned the relationship between the MMP-9-1562C/T

polymorphism and chronic periodontitis, also the same study mentioned that the MMP-9 is the most abundant MMPs in periodontal tissues reflecting periodontal disease severity, progression, and treatment response.

Conclusion

Our study concludes that the salivary Matrix metalloproteinases (MMPs) determination can definitely be used as a biomarker for the diagnosis and progression of periodontal diseases. It may also facilitate the better treatment of periodontal disease and the assessment of periodontal conditions in medical and research settings.

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Conflict of Interest: None to declare.

Ethical Clearance: "All experimental protocols were approved under the Al-Nahrain University and carried out in accordance with approved guidelines".

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