Association between MMP-1 Gene Promoter Genetic Variation and Chronic Periodontitis Susceptibility in the Iranian Population

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Abstract

Background: A genetic variation was described in the promoter region of the human MMP-1 gene, and this genetic variation has been associated with risk of inflammatory diseases. We aimed to evaluate the association between the MMP-1 promoter gene genetic variation (1G/2G at -1607) and incidence and severity of chronic periodontitis (CP) susceptibility in an Iranian population. Methods: In this analytic cross-sectional study, 100 Iranian subjects were allocated to case (with CP, n=50) and control (with normal periodontium, n=50) groups. Clinical indices (plaque index, clinical attachment loss, bone loss, and probing pocket depth) were measured before genetic analysis. Genomic DNA was obtained from whole blood samples. MMP-1 promoter genetic variations (-1607) were genotyped using PCR-RFLP method and the clinical and genetic data were analyzed with t, Chi-square, Mann-Whitney, and Fisher's exact tests. Results: Genotype analyses revealed no significant differences in the distribution of MMP-1 promoter (G1/G2) genotype (at -1607 locus) between the two groups (P=0.495). There was significant correlation between MMP-1 genotype 1G/2G with clinical attachment loss and age in the CP group (P=0.046 and 0.047 respectively), but no significant correlation was found between MMP-1 genotype 1G/2G and other periodontal indices (bone loss, probing pocket depth and plaque index). Conclusion: MMP-1 genetic variation does not increase the susceptibility to chronic periodontitis in patients CP, while there is an association between attachment loss and MMP-1 genetic variation.

Key Words: Genetic variation, Matrix metalloproteinase, Gene-promoter, Chronic periodontitis

Introduction

Periodontitis is a common and complex infectious disease in adults older than 35. It is classified as chronic (the most frequent type), aggressive, and as a manifestation of a systemic disease [1]. In this disease, the inflammatory process causes tissue damage and supporting dentation structures, including alveolar bone [2]. Considering the association of periodontal diseases with systematic diseases, such as obesity, diabetes mellitus type 2, pulmonary diseases, and cardiovascular diseases [3-5], the significance of diagnosis and management of periodontal diseases, especially chronic periodontitis (CP), is not only due to dental loss, but also the systemic responses of tissue degeneration in the body [6,7]. The host immune responses are usually activated against microbial agents, which is dominated by the genetic profile of the individual [8].

The influence of genetics on the activation of the inflammatory process in CP has been well established [9]; Matrix metalloproteinase (MMP) is a proteolytic enzyme [10], which can degrade extracellular matrix macromolecules, including collagen, macrophage, and lymphocytes [11]. It has been confirmed that gingival fibroblasts can cause extracellular matrix destruction by overexpression of MMPs by inflammatory markers interleukin-1 (IL-1), and tissue necrosis factor-alpha (TNF-) [12]. Single nucleotide genetic variation of MMP-1 gene in the 1G/2G promoter region of –1607 bp of MMP-1 gene has been found to be associated with increased risk of generalized aggressive periodontitis [12]. Although some studies have suggested the role of MMP-1 in extracellular matrix destruction [13], some other studies have shown no effect [14].

Due to the discrepancy in the results of previous studies that varies due to ethnical differences [15], as well as the significance of this health issue, especially in the Iranian population, which has not been studied well, we aimed to evaluate the association between the MMP-1 promoter gene genetic variation and incidence and severity of CP in an Iranian population.

Materials and Methods

Study design

In this analytic descriptive case-control study, 100 Iranian patients who referred to the Periodontology ward of Isfahan Hospital of Dentistry, University of Isfahan, Iran, were enrolled by convenient sampling method. The study sample size was calculated based on beta error of 5% and power of 80% according to previous studies. The participants were divided into case (n=50) and control (n=50) groups. Patients were recruited into the case group, when they had established chronic periodontitis in the primary examination and had at least one clinical attachment level (CAL) without any systemic disease and patients with normal periodontium without any signs of attachment loss (AL) and periodontal pocket depth (PPD) were recruited into the control group. Patients who had systemic diseases affecting periodontal status, including diabetes mellitus, rheumatoid arthritis, and pregnancy, as well as patients receiving any drug or treatment that affect periodontitis, including smoking, non-steroid anti-inflammatory drugs (NSAIDs), bisphosphonates, and hormones were excluded from the study.

The data collected prior to genetic analysis included demographic characteristics, medical history, physical examination of clinical periodontal indices, including CAL,

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PPD, plaque index (PI) and bone loss (BL). AL was measured by Williams's periodontal probe (Friedy HU, Chicago, USA) as the distance between CEJ to the pocket depth. All measurements were performed by one trained personnel in four points of mesiobuccal, midbuccal, distobuccal, and midlingual areas. The probe depth was recorded as the distance measured from the pocket depth to the gingival margin. Panoramic radiography was used to measure the amount of BL, in which normal bone height was assumed 2 mm below CEJ and bone loss was measured compared to normal height and the results were recorded as percentage. To determine the person's dental hygiene, PI was used, which was designed by Sillness and Loe (1964) who scored PI from 0 to 3 and each tooth would receive a score from addition of these scores and PI would be calculated by dividing the sum of scores into the number of person's teeth.

Then, genomic DNA was obtained from whole blood samples; from each patient, 5 cc peripheral blood sample was taken and kept in phalcon tubes containing EDTA. MMP-1 promoter genetic variations (at -1607 locus) was genotyped using polymerase chain reaction-restriction length polymerization (PCR-RFLP) method. DNA was extracted by Salting method from cells and separated according to the company's instructions. Two forward and backward primers were designed to determine the start and end parts of DNA meant to reproduce. The PCR primers sequencing for the -1607 locus of MMP-1 is demonstrated in *Table 1*.

Table 1. *PCR primers sequencing, restriction enzyme, and the separated DNA fragment.*

Fragment(bp)	Restriction enzyme	Primer (5'-3')	
118(29 + 89)	Xmnl(37°C)	F:5-TCG TGA GAA TGT CTT CCC ATT-3'	MMP-1-1607
		R: 5'-TCT TGG ATT GAT TTG AGA TAA GTG AAA TC-3'	

According to the objectives of the study, the prevalence of genetic variation of MMP-1 was compared between the two groups of case and control and the association of MMP-1 with prevalence and severity of CP was determined.

Ethical considerations

The protocol of the study was approved by the Ethics Committee of Isfahan University. Before starting the sample collection, the design and objectives of the study were explained to all participants and written informed consent was obtained from those who were willing to participate in the study and they were assured that they were free to leave the study whenever they wished to. The analysis was performed anonymously and the patients' information was kept confidential in all phases of the study.

Statistical analysis

The clinical and genetic data were analyzed with T –Test, chi –square, Mann Whitney and Fisher exact test. Statistical analysis was performed using SPSS 16.0 software. P-values less than 0.05 were considered statistically significant.

Results

Among all participants, 48% were male and 52% were female. Sex distribution of the patients was not statistically different between the groups (P=0.423). In addition, mean age of patients was not statistically different between the case and control groups (35.6 ± 10 vs. 33.6 ± 11.4 years, P=0.364). Mean age of patients with genotype 1G/2G was 21 ± 4.2 years in the case group and 35 ± 10.65 years in case group, which was significantly different (P=0.047).

Among all samples, 98% had the genotype 1G/1G (normal) and 2% 1G/2G; among the cases, 48 patients (96%) had normal genotype and only 4% had the genotype 1G/2G and all members of the control group had a normal genotype. But the distribution of 1G/2G had no significant difference between the two groups (P=0.495). The details are demonstrated in *Table 2*.

Table 2. *Statistical tests for sex distribution, mean age and distribution of 1G/2G in different groups.*

	Two groups	Statistical test	P-value
Sex distribution	normal and CP	Chi-square	0/423
Mean age	normal and CP	t-test	0/364
Mean age	1G/1G and 2G/2G	Mann Whitney	0/047
Distribution of 1G/2G	normal and CP	Fisher exact	0/495

In the group with CP, 24 patients had mild CP, 17 had moderate CP, and 9 had severe CP. All the patients with mild CP had the normal genotype, and one case of moderate CP (5.9%) and one case of severe CP (11.1%) had 1G/2G genotype. Mean periodontal CAL was 6 ± 1.41 in patients with 1G/2G genotype and 3.7 ± 1.5 in patients with 1G/1G genotype, which was significantly different (P=0.046). But mean BL, PPD, and PI was not significantly different between patients with the two genotypes (P=0.552, 0.688, and 0.753, respectively). The details are demonstrated in *Table 3*.

Table 3. *The mean periodontal indices and age in CP group with the genotype 1G/2G compared to 1G/1G.*

Periodontal index	Genotype	Mean ± standard deviation	P-value	statistical test
CAL	1G/2G	6 ± 1.41	0.046	T-test

	1G/1G	3.72 ± 1.54		
BL	1G/2G	12.5 ± 3.53	0.552	T-test
	1G/1G	9.7 ± 6.45		
PPD	1G/2G	4 ± 0	0.688	T-test
	1G/1G	4.3 ± 1.15		
PI	1G/2G	42.5 ± 3.53	0.753	T-test
	1G/1G	46.1 ± 15.9		
Age	1G/2G	21 ± 4.2	0.047	Mann Whitney
	1G/1G	36.2 ± 9.7		

The mean age of patients with the two genotypes was statistically different (P=0.046). CAL was associated to 1G/2G genotype, even after adjustment for age.

Discussion

The influence of inflammatory responses, ruled by genetic and hereditary pattern, has changed the view towards periodontitis. In the present study, comparison of patients with CP with age and gender-matched control group, showed CAL as the only periodontal index associated to 1G/2G genotype. The prevalence of 1G/2G genotype was 2% among total patients: 4% of the CP group and 0% in the control group (P=0.495).

Different studies have reported dissimilar results regarding the association of MMP-1 with chronic periodontitis. De Souza et al. have shown a significant association between MMP-1 and severe CP in a Brazilian non-smoker sample [16]; the same team have later identified that although the frequency of 2G genotype is more in CP, its distribution is same between CP and control and is thus not associated with susceptibility to the disease [14], which was consistent to the results of the present study. They have interpreted the discrepancy to be as a result of methodologic differences, including larger sample size. Furthermore, they have suggested that overexpression of MMPs in mRNA might not necessarily mean increased functional activity. Izakovičová Hollá has evaluated this association in 329 Caucasian subjects in Czech population and have associated the −1607 1G allele with CP among non-smokers and have concluded small effect of MMP-1 genetic variations on the pathogenesis of CP [17]. Cao et al. have demonstrated a three-fold increase in the prevalence of severe CP with 2G allele in a Chinese population [18]. Itagaki et al. have established no difference in the distribution of MMP-1 and -3 in a Japanese population and have suggested that MMP-1 expression is highly influenced by inflammatory responses rather than genetics [19]. They have interpreted the difference among studies due to the ethnical/racial differences of allele expression. Ustun and colleagues have also reported no significant association for MMP-1 −1607 1G/2G genetic variation with susceptibility to periodontitis in a Turkish population [20], which was consistent with the results of the present study. The authors anticipate that the discrepancy among studies is predominantly caused by methodologic differences of the studies beside the differences in the demographic characteristics of participants, including racial differences and medical history, such as smoking and underlying diseases, which obviously affect the results. In addition, it is believed that assessing the complex disease of periodontitis requires a large investigation that takes all host-microbial factors into account and evaluating one factor cannot provide accurate results. To achieve an unbiased result, it is necessary to investigate all factors affecting the incidence and severity of CP, including having a specific allele, transcription factors, translation factors, and bioactive materials' interactions.

The strengths of the present study included assessing this race-related issue in a population, in which it has not been previously investigated; however, the high cost of genetic investigations and laboratory assessments that required time and budget limited the study sample size. Besides, it was difficult to convince patients to undergo a blood sample and accept participation in the study. The present study was also limited in some aspects, including assessing patients referring to one center, while a population-based study with large sample size is required to assess all the effective factors, especially in Iranian population, where this important health issue has been neglected.

As far as we are concerned, no other Iranian study has evaluated this issue; therefore, according to the results of the present study, it can be concluded that in Iranian population, CP is not associated to the genetic variation of MMP-1, but the amount of the disease advancement and the resulting degradation is associated with MMP-1. It can be also concluded that CAL is more dominantly controlled by genetics, rather than regional factors, such as plaque.

Conclusion

MMP-1 genetic variation does not increase the susceptibility to chronic periodontitis in patients CP, while there is an association between attachment loss and MMP-1 genetic variation.

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