

Original Article

Association of macrophage migration inhibitory factor gene polymorphisms with chronic periodontitis in a South Eastern Iranian population

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ABSTRACT

Background: Macrophage migration inhibitory factor (MIF) is a key proinflammatory mediator. It plays a vital role in immune response against the oral disease. MIF is a regulator of innate immunity, and bacterial antigens can stimulate serum level of this protein. In experimental gingivitis, the expression level of MIF increases and this increment positively correlates with oral plaque index. The single nucleotide polymorphisms in the gene encoding the MIF protein can control the function of MIF. The aim of the present study was a clarification of the associations between *MIF-173 G/C*, *MIF 95 bp*, and *189 bp insertion/deletion (I/D)* polymorphisms and chronic periodontitis (CP) compared with healthy controls.

Materials and Methods: This case-control study was carried out on 210 CP patients and 100 normal subjects. *MIF-173 G/C* and *MIF 95 bp* and *189 bp I/D* polymorphisms were genotyped, using polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) and PCR, respectively. Allele and genotype frequencies of the variants were compared between patients and controls using Chi-square test. The value of $P < 0.05$ was considered statistically significant.

Results: The study findings showed that *MIF-173 G/C* polymorphism, especially the C allele increased the risk of CP. The 95-bp *I/D* polymorphism was not associated with CP and the 185-bp *I/D* variant was not polymorphic in our population.

Conclusion: Therefore, *MIF-137 G/C* variant increased the risk of CP in the South East of the Iranian population. In other words, polymorphisms in MIF gene influence clinical outcome of CP infection and influence the susceptibility to disease. Further studies with larger sample sizes and different ethnicities are required to validate our findings.

Key Words: Chronic periodontitis, gene, Macrophage Migration-Inhibitory Factors, migration inhibitory factor, polymorphism

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INTRODUCTION

Chronic periodontitis (CP) is a widespread oral disease mainly seen in adults. Individuals with CP are

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encountered with the destruction of periodontal tissue, leading to attachment loss and eventually alveolar bone destruction and tooth loss.^[1-3]

Excessive productions of inflammatory cytokines and cytokine gene polymorphisms have been shown that were associated with periodontitis.^[4,5] Different allelic variants can act as protective or risk factors for the disease.^[6]

In periodontal disease, the inflammatory response is initiated after toll-like receptor activation that leads to the production of host mediators involved in the inflammatory immune response. Proinflammatory cytokines induce tissue destruction through the production of mediators that amplify and maintain the inflammatory response. It causes the production of osteoclastogenic factors and lytic enzymes.^[7]

Macrophage migration inhibitory factor (MIF) is a proinflammatory protein encoded by the MIF gene. MIF was described as a product of activated T-cells. MIF is a regulator of innate immunity and bacterial antigens can stimulate serum level of this protein.^[8] MIF binds to other immune cells through CD74 to trigger an acute immune response; hence, MIF can be classified as an inflammatory cytokine.^[9] Previous studies showed that during experimental gingivitis, the expression level of MIF is increased and this increment positively correlates with oral plaque index.^[9,10]

MIF has been involved in some diseases such as rheumatoid arthritis, which shares pathogenic features with periodontal disease.^[11] Genetic studies have shown that polymorphisms in cytokine genes are associated with the increased susceptibility to some chronic inflammatory diseases such as periodontitis.^[8,12]

The gene encoding MIF contains three short exons, separated by two introns, and spans approximately 1 kb on chromosome 22q11.23. Two polymorphisms have been associated with the severity of systemic-onset juvenile idiopathic arthritis (SJRA) and rheumatoid arthritis, a G-to-C single nucleotide polymorphism (SNP) at position -173 (rs755622) and a *CATT*-tetranucleotide sequence repeated 5–8 times at position-794.^[9]

Donn *et al.*^[13] reported a G-to-C transition at position -173 of the *MIF* gene in patients with SJRA. They found that the risk of SJRA increased in the individuals possessing the *MIF-173C* allele. Furthermore, De Benedetti *et al.* found that increased

synovial fluid and serum levels of MIF, poor response to glucocorticoid treatment, the durability of active disease and a weak functional outcome was associated with the *MIF-173C* allele.^[14]

Li *et al.*^[15] showed that the expression of MIF factor may be associated with the periodontal status of the individual. Donn *et al.*^[13,16] showed that -173C allele is associated with increased production of MIF protein.

MIF gene can have SNPs in exons and introns.^[9] The rs71891258 (*MIF* 95 bp) and rs7167333 (*MIF* 189 bp) are two biallelic insertion/deletion (I/D) polymorphisms in introns of MIF. There is a little information about the relationship of these two polymorphisms and the regulation of expression of the MIF gene. For the first time, in this study, we investigated the relationship between *MIF* 95 bp and 189 bp I/D polymorphisms and CP.

As the frequency of many genetic alleles varies between ethnic groups and there is no report about the relationship between MIF gene SNPs and CP, the aim of this study was to investigate the effect of *MIF-173G/C*, 189- and 92-bp I/D SNPs as host susceptibility risk factors in CP in a Southeastern Iranian population.

MATERIALS AND METHODS

Patients and control subjects

The study was approved by the Institutional Ethics Committee of the Zahedan University of Medical Sciences (No. 6203), Zahedan and carried out in Dental College and Infectious Diseases and Tropical Medicine Research Center, Zahedan, Iran. One hundred systemically healthy and two hundred and ten patients from the Southeast region of Iran were included in this case-control study. Patients were included in the study after obtaining informed consent. The patients were diagnosed according to the criteria of the International Workshop for classification of periodontal diseases and conditions.^[17] The disease diagnosis was based on physical examination, medical and dental history, probing depth (PD) and assessment of clinical attachment loss. Probing was performed at six sites around each tooth using the WHO periodontal probe and recording the maximum values, tooth mobility, and radiographs. The loss of alveolar bone was determined radiographically. Controls were unrelated healthy individuals selected from patients referring to the Dentistry Clinic for reasons

other than periodontal disease and were matched for age, ethnicity, and gender. All the subjects were nonsmokers and had at least twenty teeth. Exclusion criteria were included: The use of anti-inflammatory drugs, orthodontic instruments, smoking, pregnancy, infections in the oral cavity, diabetes, rheumatoid arthritis, lupus erythematosus, HIV, hepatitis, cardiovascular disease, and other systemic diseases that affect the periodontal condition.

Genotyping of polymorphisms

Two milliliters of peripheral blood samples were collected in Na-EDTA tubes. Genomic DNA was extracted using the salting-out method.^[5] Genotyping of the *MIF-173 G/C* polymorphism was determined by employing polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) method.^[18] The MIF genomic sequence was obtained from the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>). The polymorphisms were searched, and primers were designed for PCR-RFLP and PCR, which is a simple and rapid technique for recognition of SNP. The primers used for genotyping of *MIF-173 G/C* as well as *MIF 95 bp* and *189 bp I/D* polymorphisms are listed in Table 1. The amplification procedure was carried out using Mastercycler gradient (Eppendorf).

Genotyping of *-173 G/C* SNP was performed using 2X Prime Taq Premix (Genet Bio, Korea). In each 0.20 ml reaction, 1 µl of each primer, 1 µl of genomic DNA (~100 ng/ml), and 10 µl of 2X Prime Taq Premix and 7-µl ddH₂O were added. PCR was performed according to the following protocol: Initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 30 s, annealing at 67°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 5 min. The PCR product (10 µl) was digested using AluI (Fermentas, Vilnius, Lithuania) restriction enzyme. The C allele was digested and produced 255 bp and 184 bp fragments while the G allele was undigested and produced a 439 bp fragment. The PCR and fragments were verified on 2% agarose gels containing 0.5 µg/ml ethidium bromide and observed under ultraviolet light (Gel Documentation, Germany) [Figure 1].

Genotyping of *MIF 95 bp I/D* and *189 bp I/D* polymorphisms produced 727 bp for both insertion alleles, 632 bp for only *MIF 95 bp* deletion allele, 538 bp for only *MIF 189 bp* deletion allele, and 463 bp for both deletion alleles. PCR was performed

Table 1: The primers sequences used for detection of migration inhibitory factor-173 G/C and migration inhibitory factor 95 bp and 189 bp insertion/deletion polymorphisms gene polymorphisms using polymerase chain reaction

Polymorphisms	Sequence (5'→3')
MIF-173 G/C	F: CTCAAACACACAAGCTCACGCATGCG R: ACCACTGTGGTCCCGCCTTTTGTGAC
MIF 95bp 189bp I/D	F: TTCATCGTAAACACCAACG R: GTTTATTTCTCCCAACAG

MIF: Migration inhibitory factor; I/D: Insertion/deletion polymorphisms

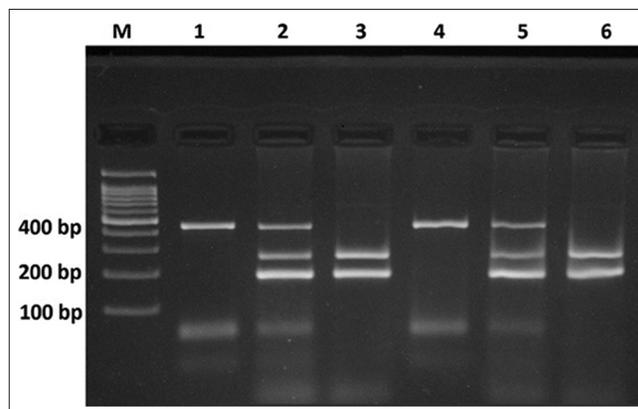


Figure 1: Representative agarose gel electrophoresis illustrating polymerase chain reaction products for the migration inhibitory factor-173 G/C polymorphism: M: DNA marker; lanes 1 and 4: Homozygous GG subject; lanes 2 and 5: Heterozygous GC subject; lanes 3 and 6: Homozygous CC subject.

in a final volume of 0.20 ml containing 1 µl of genomic DNA (~100 ng/ml), 1.5 µl of each primer and 10 µl of 2X Prime Taq Premix (Genet Bio, Korea) and 6 µl ddH₂O. PCR was performed in conditions as follows: 95°C for 5 min; 95°C for 30 s, 62°C for 30 s, 72°C for 30 s, 30 cycles, and a final step at 72°C for 5 min to allow for the complete extension of all PCR fragments. The PCR products were electrophoresed on 2% agarose gel containing 0.5 µg/ml ethidium bromide [Figure 2].

Statistical analysis

Statistical analysis was performed using the statistical software package SPSS 20 (SPSS Inc., Chicago, IL, USA). The association between genotypes and CP were assessed using computing the odds ratio (OR) and 95% confidence intervals (95% CIs) from logistic regression analyses. Quantitative data were presented as mean ± standard deviation. Comparison of quantitative variants between two groups was assessed using Student's *t*-test. The frequencies of the alleles and genotypes were analyzed using the Chi-square test. Allele frequencies were calculated by

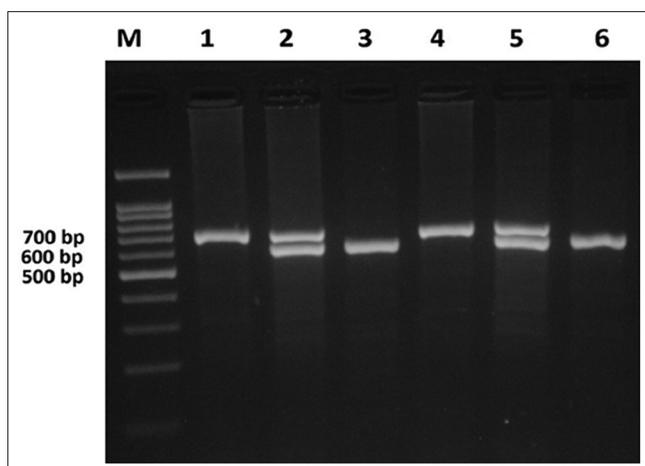


Figure 2: Representative polymerase chain reaction products resolved by agarose gel electrophoresis to detect the presence or absence of the 95 bp insertion/deletion of migration inhibitory factor gene. M, DNA marker; lanes 1 and 4, insertion/insertion; lane 3 and 6, deletion/deletion; lanes 2 and 5, insertion/deletion.

the gene counting method. The value of $P < 0.05$ was considered statistically significant.

RESULTS

Subject characteristics

Differences in demographic measurement and clinical parameters between groups are depicted in Table 2. The CP group exhibited a significantly greater mean of periodontal PD (5.58 ± 0.63 mm vs. 1.50 ± 0.86 mm), clinical attachment level (5.44 ± 0.58 mm) and a higher percentage of sites with bleeding on probing ($85.86\% \pm 3.68\%$) than the control group ($P < 0.05$). The mean age of healthy subjects and CP patients were 29.22 ± 3.60 and 28.33 ± 5.76 , respectively. There was no significant difference between cases and controls regarding gender, age, and ethnicity ($P > 0.05$).

Genotype and allele frequencies

The frequencies distribution of genotypes and allele carriage of the *MIF-173 G/C*, 95 bp I/D in subjects with CP and control are described in Table 3. No deviation from Hardy–Weinberg equilibrium was observed within either group ($P > 0.05$).

The genotypic and allelic frequencies were statistically different between CP and healthy subjects, and the risk of CP was higher in individuals with GC genotype in comparison to those with GG genotype (OR = 2.25, 95% CI = 1.10–54.57, $P = 0.026$). In addition, the frequency of CC + GC genotypes in CP patient was more than the control

Table 2: Demographic parameters of chronic periodontitis patients and control group

Parameters	CP (n=210)	Control (n=100)	P
Age (years)	28.33±5.76	29.22±3.60	0.159
Gender			
Male	115 (54.8)	48 (48.0)	0.265
Female	95 (54.2)	52 (52.0)	
Ethnicities			
Sistani	82 (39.2)	42 (42.0)	0.186
Baluch	71 (33.8)	24 (24.0)	
Others	57 (27.1)	34 (34.0)	
Clinical parameters			
BOP index (%)	85.86±3.68	-	
Mean PD (mm)	5.58±0.63	1.50±0.86	
CAL (mm)	5.44±0.58	-	

CP: Chronic periodontitis; BOP: Bleeding on probing; PD: Probing depth (mm); CAL: Clinical attachment level (mm)

group and this difference was significant ($P = 0.010$). Furthermore, the frequency of C allele was significantly higher in CP patients. Therefore, this allele could be a risk factor for susceptibility to CP (OR = 2.30, 95% CI = 1.28–4.14, $P = 0.006$) in comparison with G allele. As shown in Table 3, we did not observe any significant difference either in the genotype distribution or in the allele frequency of the *MIF* 95 bp I/D SNP between patients and controls. The combination of different genotypes of *MIF-173 G/C* and *MIF* 95 bp I/D SNPs generates 9 haplotypes as shown in Table 4. The findings showed that haplotypes were not significantly different between patients with CP and controls ($P = 0.071$).

DISCUSSION

In this study, we investigated the impact of *MIF* gene SNPs on CP. The findings indicated that there were statistically significant differences in frequencies of genotypes and allele carriage of the *MIF-173 G/C* between the CP patient and normal subjects. Furthermore, in our study, no statistically significant difference was seen between the CP patients and controls, with respect to genotypes of *MIF* 95 bp I/D SNP.

MIF has been detected in gingival crevicular fluid and periodontal tissues.^[10] Genetic studies suggest that SNPs in cytokines or their receptors are associated with an increased risk in the progression of chronic inflammatory disorders such as periodontitis.^[19] In addition, the results of studies on *MIF* gene polymorphisms have expressed conflicting results in patients with asthma, rheumatoid arthritis and systemic lupus erythematosus.^[20,21]

Table 3: The frequency of genotypes and alleles of migration inhibitory factor-173 G/C, migration inhibitory factor 95 and 189 bp insertion/deletion polymorphisms gene in 210 patients and 100 healthy subjects

MIF polymorphisms	CP, n (%)	Control, n (%)	OR (95%CI)	P
-173 G/C				
GG	155 (73.8)	87 (87.0)	1	-
GC	44 (21.0)	11 (11.0)	2.25 (1.10-4.57)	0.026
CC	11 (5.2)	2 (2.0)	3.09 (0.67-14.25)	0.149
GC + CC	55 (26.2)	13 (13.0)	2.38 (1.23-4.59)	0.010
Allele				
G	354 (84.3)	185 (92.5)	1	-
C	66 (15.7)	15 (7.5)	2.30 (1.28-4.14)	0.006
95 bp I/D				
I/I	106 (40.5)	58 (58.0)	1	-
I/D	74 (35.2)	34 (34.0)	1.19 (0.71-2.00)	0.508
D/D	30 (14.3)	8 (8.0)	2.05 (0.88-4.77)	0.095
I/D + D/D	104 (49.5)	42 (42.0)	1.36 (0.84-2.19)	0.215
Allele				
I	286 (68.1)	150 (75.0)	1	-
D	134 (33.9)	50 (25.0)	1.41 (0.96-2.06)	0.079
189 bp I/D				
I/I	210 (100)	100 (100)	-	-
I/D	0	0	-	-
D/D	0	0	-	-

I/D: Insertion/deletion polymorphisms; CP: Chronic periodontitis; OR: Odds ratio; CI: Confidence interval; MIF: Migration inhibitory factor

Table 4: Haplotype frequencies in chronic periodontitis (case) and normal subjects (control)

Haplotypes	CP group, n (%)	Control group, n (%)
II/CC	1 (0.5)	1 (1.0)
II/GG	76 (36.2)	47 (47)
II/GC	29 (13.8)	10 (10.0)
DD/CC	4 (1.9)	1 (1.0)
DD/GG	22 (10.5)	6 (6.0)
DD/GC	4 (1.9)	1 (1.0)
ID/CC	6 (2.9)	0
ID/GG	57 (27.1)	34 (34)
ID/GC	11 (5.2)	0
Total	210 (100.0)	100 (100.0)

$\chi^2=14.43$; $P=0.071$. CP: Chronic periodontitis

The rs755622, rs71891258, and rs7167333 are located in the promoter of the MIF gene. The frequency of I or D alleles of the 95-bp I/D SNP in the patient and control groups indicated no significant correlation between allele and CP. Although the frequency of alleles and genotypes of 95-bp I/D SNP were different in patients with CP, the number of samples with various genotypes was not enough to be considered statistically significant and the same situation was seen in the control group. The 189-bp I/D SNP was not polymorphic in our population. All participants had genotype II. We did not see any ID and/or DD genotypes in any of groups.

It is supposed that MIF functions as a cytokine to promote cytokine expression and control the recruitment and migration of macrophages to the site of inflammation^[22,23] and produce an essential response in the presence of pathogens.^[24] Furthermore, it has been shown that polymorphisms of the human MIF gene have been associated with increased susceptibility to inflammatory diseases.^[21,25]

We have already reported the association between CP and cytokines SNPs^[5,26-29] in Southeast of Iran. Moreover, there are two polymorphisms in MIF gene that are associated with MIF transcription level: A tetranucleotide *CATT* repeat at position - 794 and a SNP at position - 173. There has been no report of an association of *MIF-173 G/C* with CP so far. Therefore, the roles of *MIF-173 G/C* polymorphism in the development of CP remain unclear.

In the present study, we found a significant association between rs755622 in MIF gene promoter and CP. The *MIF-173 GG* genotype was significantly associated with a decreased risk for the development of CP. Donn *et al.* have found that the -173C allele creates an activator protein 4 transcription factor binding site and MIF expression differed among *G-173C* genotypes in a cell-type manner. The -173C allele increase the promoter activity in a T lymphoblast

cell line and seemed to promote the production of MIF,^[16] subsequently *-173C* allele may produce the development of CP. Furthermore, in many studies, the *-173G* allele (75%–90%) was more common than the *-173C* allele (15%–20%) in healthy subjects from various countries.^[30–33] This study has shown a significant association between carriage of the *MIF-173C* allele and CP. The G/C + C/C genotype was significantly associated with an increased risk for the development of CP.

In accordance with our study, some researchers reported that *MIF-173C* allele may be associated with susceptibility to some inflammatory diseases such as ulcerative colitis,^[34] rheumatoid arthritis,^[35] psoriasis,^[36] and chronic gastritis.^[37] In these reports, higher levels of human circulating MIF have been found in carriers of the *-173C* allele. Furthermore, the significant association of the *-173C* allele with increased risk of above-mentioned diseases would underlie the proinflammatory effect of elevated circulating MIF protein. On the other hand, there are reports that not observed the association between *MIF G-173C* and chronic inflammation such as ulcerative colitis.^[38]

In the current study, we hypothesized that these two SNPs in MIF gene might be associated with the development of CP. Although there is no clear relationship between these SNPs and the transcriptional regulation of the MIF gene, it may be that the *-173C* allele promotes the production of MIF. Our data show an association of MIF gene SNPs and susceptibility to CP, but the mechanism of this relationship is unknown. It is possible that the MIF-SNPs and other susceptibility factors together play an important role to increase risk of CP. In the present study, none of the subjects was smokers, so simultaneous investigation of genotypes and smoking and its association with CP was not possible. To clarify the role of these SNPs to the development of CP, it is important to study genotype distributions between different races and various geographic populations because allele frequencies can vary in different populations. In addition, according to researchers, several different genes such as tumor necrosis factor alpha, interferon gamma, and interleukin-10 are involved in the risk of periodontitis. Therefore, this is necessary to carry out various genetic tests to determine the relationship between periodontal disease and the genotypes of several cytokines.^[5,19,39]

CONCLUSION

Functional polymorphisms of the MIF gene promoter may be associated with the risk of CP inflammation and with the subsequent development of periodontal destruction. Our results indicate that *MIF-173 G/C* polymorphism is associated with increased risk of CP. The small sample size for rare alleles affects the relationship between genotypes and clinical symptoms.^[40] Given that 95-bp and 189-bp I/D polymorphisms were not studied previously, it is difficult to make an accurate decision about them. So in future studies, we can find out the relationship between CP and 95-bp and 189-bp I/D polymorphisms properly with increasing the sample size, and the investigation of the possible relationship between these SNPs and other factors such as age, gender, ethnicity, diet, personal habits such as smoking. Further studies with large sample size will be needed to confirm our finding.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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