

Original Article



Association Between MMP-2 and MMP-9 Promoter Polymorphisms with Gastritis and *Helicobacter Pylori* Infection Susceptibility

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Abstract

Introduction: *Helicobacter pylori* is the main cause of gastritis. Degradation of the extracellular matrix is associated with gastric mucosal injury and is facilitated by matrix metalloproteinases. This study aimed to investigate the association between functional polymorphisms in the promoters of the *MMP-2* and *MMP-9* genes and gastritis and *H. pylori* infection.

Methods: The case-control study included 132 patients with gastritis and *H. pylori* infection, 98 patients uninfected with *H. pylori*, and 103 healthy individuals. After genomic DNA extraction, genotypic frequencies in the three groups were determined using the Tetra Primer ARMS-PCR method. Independent t-test, chi-square test, and logistic regression analysis were used for data analysis.

Results: A statistically significant difference was observed between the *MMP-9* -1562 T/T genotype and an increased risk of gastritis ($P=0.007$) and *H. pylori* infection ($P=0.001$) among the studied groups. Additionally, a notable correlation was found between gastritis ($P=0.004$) and *H. pylori* infection ($P=0.006$) in individuals with the *MMP-2* -1306 T/T polymorphism compared to the control group. Based on the results, having the T allele at positions -1562 and -1306 increased the likelihood of gastritis and *H. pylori* infection.

Conclusion: Our findings revealed that *MMP-9* -1562 C/T and *MMP-2* -1306 C/T polymorphisms may be associated with an increased risk of gastritis and *H. pylori* infection. However, further studies on larger and diverse populations are required to generalize these results.

Keywords: Gastritis, *Helicobacter pylori*, Polymorphism, *MMP-9*, *MMP-2*

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Introduction

Gastritis is one of the most common digestive diseases, which involves inflammation or irritation of the inner lining of the stomach. In addition, it can occur acutely or chronically and plays an important role in the development of stomach diseases. Globally, gastritis affects 50.8% of the population in developing countries (1). Although the exact causes of gastritis are not perfectly understood, environmental and genetic factors play a role. One of the most common types of chronic gastritis is pyloric gastritis, which is caused by infection with *Helicobacter pylori* (2, 3). *H. pylori* is a Gram-negative, microaerophilic bacillus. Infection with this bacterium leads to chronic inflammatory reactions and tissue damage in the gastric mucosa. These inflammatory reactions are caused by several bacterial products, such as vacuolating cytotoxin A and cytotoxin-associated gene A (4, 5). The immune response to *H. pylori* infection starts with an increase in cytokines and chemokines, which stimulate gastric epithelial cells to produce matrix-degrading enzymes for regenerating epithelial tissue (5, 6). Moreover, degradation of the extracellular matrix (ECM) is closely related to gastric mucosal injury, a process that

is facilitated by the activity of matrix metalloproteinases (MMPs), a type of endopeptidases. MMPs are a family of similar, zinc-dependent enzymes that are involved in the digestion of many ECM components, cell proliferation, and inflammation, and participate in biological processes, such as embryonic development, angiogenesis, and wound healing (7). Normally, these enzymes are expressed in low amounts; however, their expression increases with the initiation and activation of destructive processes, including cancer cell invasion, metastasis, and inflammation. Likewise, MMPs are divided into 6 groups based on differences in domain structure and substrate specificity: collagenases, gelatinase, stromelysin, matrilysins, membrane-type MMPs, and other MMPs (8). Among the MMP family, gelatinases, including *MMP-2* (gelatinase A with 72kDa) and *MMP-9* (gelatinase B with 92kDa) are capable of degrading collagen types IV and V, gelatin, and fibronectin in the ECM (9). Furthermore, these enzymes are produced and released by gastric and duodenal epithelial cells, macrophages, and neutrophils (10). The *MMP-9* gene is located on chromosome 20q12.2. It has been found that there is a single-nucleotide polymorphism at position -1562 C/T in the promoter

region of the *MMP-9* gene, which can have a functional effect on the transcription and expression of this gene. The *MMP-2* gene is located on chromosome 16q13.21. A functional single-nucleotide polymorphism located in the *MMP-2* gene promoter at position -1306 C/T changes the amino acid sequence from alanine to valine at position 429 of the protein (11). Studies have shown that the expression of these enzymes increases in certain diseases, such as cancer. This is why they can be recognized as biomarkers in various fields, including early diagnosis and disease control (12). Given the increasing prevalence of gastritis and *H. pylori* infection as the main cause of its occurrence and the role of MMPs in ECM remodeling, this study aims to investigate the association between functional polymorphisms in the promoter region of *MMP-2* and *MMP-9* genes with *H. pylori* infection and gastritis in the northern Iranian population.

Materials and Methods

Sample Collection

This case-control study involved 132 patients with *H. pylori*-infected gastritis (group 1), 98 individuals with gastritis but no infection (group 2), and 103 healthy individuals as the control group (group 3). All samples from patients and controls were collected from the Guilan Gastroenterology and Hepatology Specialized Clinic (Rasht) and the Sari Gastroenterology and Hepatology Research Center between March and October 2023. Upon referral to endoscopy centers, two biopsy samples were taken from each individual during endoscopy by a gastroenterologist (after initial diagnosis and confirmation), one of which was sent to pathology and the other to the genetics laboratory (in sterile vials). The samples were grouped based on endoscopic, pathological, and laboratory findings. In addition, individuals were placed in the first group if their gastritis and their infection with *H. pylori* were confirmed by pathology results (with hematoxylin and eosin staining) and by urea breath and serological (immunoglobulin G antibodies) tests, respectively. The second group included individuals whose gastritis was positive, while *H. pylori* infection was negative (based on the diagnostic methods mentioned for the first group). The third group (the control group) individuals were selected from healthy volunteers without *H. pylori* infection and gastritis in the same age range and

geographical location as the patients. Informed consent forms were obtained from all patients to participate in the study according to the Declaration of Helsinki. Then, patient history, including age, gender, place of residence, and severity of disease, was collected based on medical records and completed questionnaires. The inclusion criteria for the first and second groups were individuals with gastritis and *H. pylori* infection and individuals with gastritis without *H. pylori*-infection, respectively. Samples with other gastric diseases (e.g., peptic ulcer and gastric cancer) were excluded from the study. It should be noted that all participants in the study were residents of Northern Iran, and non-natives were excluded from the study. Gastric biopsy samples for genomic DNA extraction were stored in sterile microtubes in a -70°C freezer until final extraction.

Deoxyribonucleic Acid Extraction

Genomic DNA extraction from a gastric biopsy was performed using a DNA extraction kit from tissue (Sambio, Korea) following its protocol. Subsequently, the quality of the extracted genomic DNA was assessed on a 1% agarose gel stained with safe stain (Yekta Tajhiz Azma, Iran). Additionally, the purity of the DNA was verified using a NanoDrop device (Eppendorf, Germany).

Detection of *Helicobacter pylori*

The diagnosis of *H. pylori* infections was confirmed using the rapid urease test, 16S rRNA polymerase chain reaction (PCR), and laboratory findings. Patients were classified as infected with *H. pylori* only if all three tests were positive. Finally, a pair of primers (TAG, Denmark) was utilized to detect *H. pylori* by PCR (Table 1).

Tetra Prime Amplification Refractory Mutation System-Polymerase Chain Reaction

The genotypes of *MMP-2* -1306 C/T and *MMP-9* -1562 C/T in both patients and controls were determined using the Tetra Primer ARMS-PCR method. Moreover, the specificity and accuracy of the primers were verified using Oligo7 software (version 7.60, USA) and the National Center for Biotechnology Information Basic Local Alignment Search Tool reference site (Table 1). Furthermore, each PCR was performed in a final volume of 25 µL including 3 µL of genomic DNA, 1 µL of each

Table 1. Characteristics of the Primers Used for *H. pylori* Detection and *MMP-2* and *MMP-9* Polymorphisms

Primer Name	Nucleotide Sequence	Products Size	Reference
H ₁ (forward) H ₂ (reverse)	5'- AGGATGCGTCAGTCGCAAGAT -3' 5'- CCTGTGGATAAGACAGGCCAGT -3'	305 bp	(13)
MMP-2 (-1306C/T)	FI: 5'-ATATTCCCAACCCAGCAGCCT-3' RI: 5'-GCTGAGACCTGAAGAGCTAAAAGTTG-3' FO: 5'-ACCAGACAAGCCTGAACCTGT CTGA-3' RO: 5'-TGTGACAACCGTCTCTGAGGA ATC-3'	211 bp 379 bp 542 bp	(14)
MMP-9 (-1562 C/T)	FI: 5'-CAGGCGTGGTGGCGCAGCCTATCAT -3 RI: 5'-TCCTGCCTCAGCCTCCCGAGTAGCTGTTG -3' FO: 5'-ATACAGCTTATTGGGCCGGGCCCATGG -3' RO: 5'-GCCAGCCGGCATCGGGCAGGGTCTATAT-3'	205 bp 253 bp 403 bp	(15)

Note. *H. pylori*: *Helicobacter pylori*.

primer, 12.5 µL of 2X Red mastermix (Amplicon, Denmark), and 5.5 µL of sterile double-distilled water. The optimal temperature conditions for performing the PCR were set in three stages: initial denaturation at 95°C for 5 minutes, followed by 35 cycles with the program 94°C (30 seconds), 61°C for MMP-2, and 66.5°C for MMP-9 (30 seconds), 72°C (45 seconds), and finally the final extension step at 72°C for 10 minutes in a thermocycler (Bio Rad, USA). Additionally, the amplified products were electrophoresed on a 2% agarose gel to assess the accuracy of the reaction. Likewise, band detection was performed using a gel documentation device (Bio Rad, USA). Ultimately, 10% of homozygous recessive and heterozygous samples were randomly selected and re-genotyped to ensure genotyping accuracy.

Statistical Analysis

Data were analyzed using SPSS software (version 26). Genotypic and allelic frequencies were assessed in the patient and control groups. The chi-square test was utilized to examine the significance of genotypic and allelic variances between the two groups. In this test, a *P*-value of less than 0.05 indicates the level of significance of the genotype differences in the study groups. Additionally, odds ratio (OR) and 95% confidence intervals (CI) were used to evaluate the potential influence of the aforementioned polymorphisms on the disease incidence.

Results

Questionnaire Information

In this case-control study, the genotype and allele frequencies of the -1306C/T MMP-2 and -1562C/T MMP-9 gene polymorphisms were investigated in three groups (based on disease and infection). The patient and control groups were balanced in terms of age and gender. In addition, the age range of all studied samples was between 19 and 72 years. In the first group, 68 (51.5%) were female and 64 (48.4%) were male, with a mean age of 45.72 ± 17.59 . Furthermore, 47 (47.9%) were male and 51 (52%) were female in the second group, with a mean age of 41.7 ± 6.2 . In the third group, 60 (58.2%) were male and

43 (41.7%) were female, with a mean age of 43.08 ± 15.95 . Based on questionnaire data, there was no significant difference in age and gender between the studied groups. Additionally, 47 (35.6%) and 85 (64.3%) in the first group, 57 (58.1%) and 41 (41.8%) in the second group, and 68 (66%) and 35 (33.9%) in the third group were urban and rural with regard to social status, respectively. In the first and second groups, 40.9% and 26.5% of individuals had a family history, respectively (Table 2).

After extracting DNA from gastric biopsies, to confirm the presence of *H. pylori*, a pair of primers was used to amplify a 305-bp fragment of the 23S *rRNA* gene in the positive control for the accuracy of the test (Figure 1).

Determining Genotypes

To determine MMP-2 -1306 C/T and MMP-9 -1562 C/T genotypes, fragments with lengths of 542-bp (control), 379-bp (C allele), and 211-bp (T allele), as well as 403-bp (control), 253-bp (C allele), and 205-bp (T allele), were amplified in both groups, respectively (Figure 2).

Several samples of PCR products were sequenced to confirm and substantiate the identified polymorphisms.

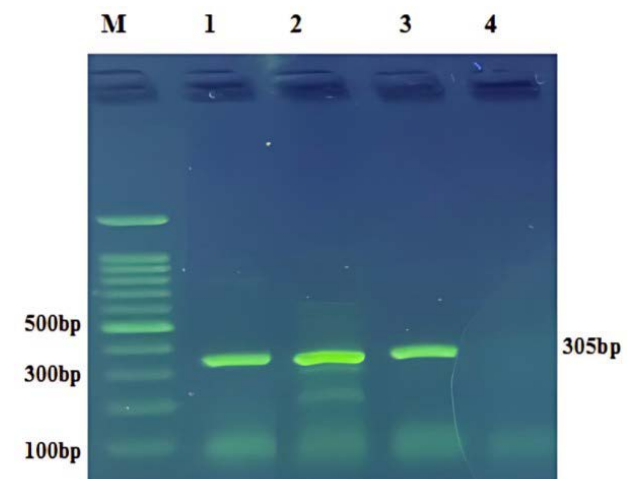


Figure 1. A 2% Agarose Gel With PCR Products for Detecting *H. pylori*
 Note. *H. pylori*: *Helicobacter pylori*; PCR: Polymerase chain reaction. Line M represents a 100 bp DNA ladder. Moreover, lines 1–3 display a 305-bp fragment indicating the presence of *H. pylori*, and line 4 denotes the absence of bacteria

Table 2. Demographic Characteristics of Patients and Controls

Characteristics	Group 1 (Gastritis With <i>H. pylori</i> infection), n (%)	Group 2 (Gastritis Uninfected With <i>H. pylori</i>), n (%)	Group 3 (Control), n (%)	<i>P</i> -Value
Gender				
Male	64 (48.4)	47 (47.9)	60 (58.2)	0.09
Female	68 (51.5)	51 (52)	43 (41.7)	
Age of onset (years, mean $\pm$ SD)	45.72 $\pm$ 17.59	41.7 $\pm$ 6.2	43.08 $\pm$ 15.95	0.13
Social status (%)				
Urban	47 (35.6)	57 (58.1)	68 (66)	0.000
Rural	85 (64.3)	41 (41.8)	35 (33.9)	
Family history				
Yes	54 (40.9)	26 (26.5)	-	0.024
No	78 (59)	72 (73.4)	-	

Note. *H. pylori*: *Helicobacter pylori*; SD: Standard deviation.

The results (Figure 3) were then analyzed using ChromasPro software (version 2.2.0 USA).

Genotype Frequencies of the -1306C/T MMP-2 Polymorphism

Table 1 provides the genotypic and allelic frequencies of the -1306C/T MMP-2 gene polymorphism. The frequency of CC, CT, and TT genotypes was 37.1%, 45.4%, and 17.4% in the first group. Likewise, the corresponding frequencies were 32.6%, 50%, and 17.3% in the second group, as well as 55.3%, 36.9%, and 7.7% in the third group, respectively. The results of the statistical analysis confirmed a significant association between the TT genotype in gastritis with *H. pylori* infection (OR=3.34, CI=1.37–8.14, $\chi^2=7.52$, $P=0.006$), gastritis without infection (OR=3.78, CI=1.47–9.74, $\chi^2=8.17$, $P=0.004$), and the control group. Additionally, the frequency of

the T allele in the desired polymorphism was 40.1% and 57.6% in the first and second groups, respectively. Based on the results, a statistically significant difference was observed between the frequency of the T allele in the first group (OR=1.88, CI=1.27–2.80, $\chi^2=10.01$, $P=0.002$), the second group (OR=2.06, CI=1.35–3.14, $\chi^2=11.63$, $P=0.001$), and the control group.

Genotype Frequencies of the -1562C/T MMP-9 Polymorphism

The participants were examined to determine the distribution of genotypes and the risk associated with the -1562C/T MMP-9 gene polymorphism. In the first group, the frequencies of CC, CT, and TT genotypes were 39.4%, 34.8%, and 25.7%, respectively. The corresponding frequencies were 37.7%, 40.8%, and 21.4% in the second group, respectively. In the third group, these frequencies

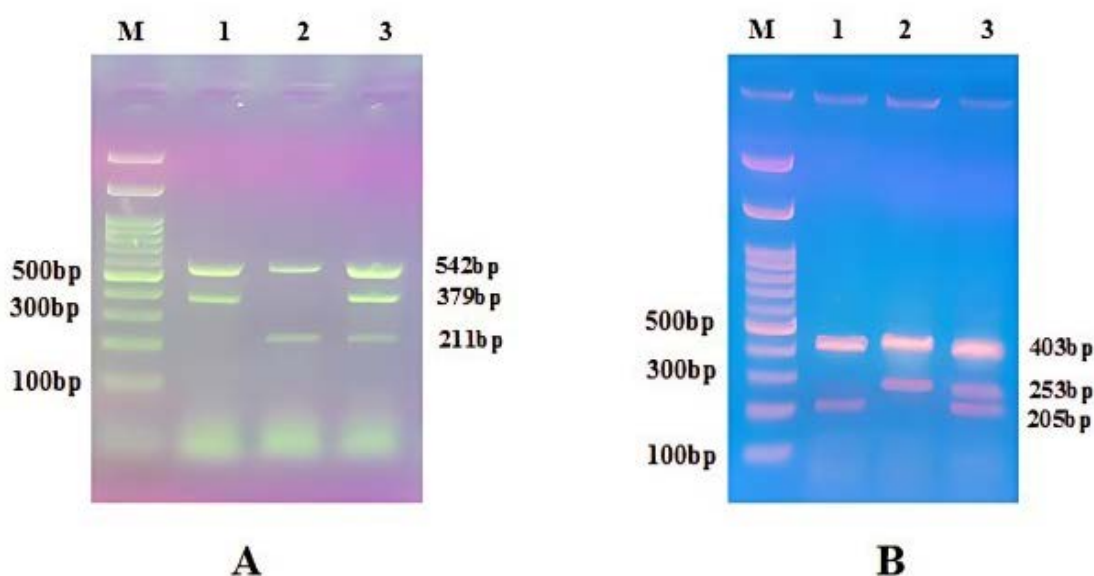


Figure 2. A 2% Agarose Gel Corresponding to ARMS-PCR Products for Determining the Genotypes of the MMP-2 -1306 C/T (A) and MMP-9 -1562 C/T (B) Polymorphisms

Note. ARMS: Amplification refractory mutation system; PCR: Polymerase chain reaction. **A:** Line M: corresponding to the 100-bp marker; Line 1: 542-bp and 379-bp fragments corresponding to the CC genotype; Line 2: 542-bp and 211-bp fragments corresponding to the TT genotype; Line 3: 542-bp and 379,211-bp fragments corresponding to the CT genotype. **B:** Line M: corresponding to the 100-bp marker; Line 1: 403,253-bp and 203-bp fragments corresponding to the CT genotype; Line 2: 403-bp and 205-bp fragments corresponding to the TT genotype; Line 3: 403-bp and 253-bp fragments corresponding to the CC genotype

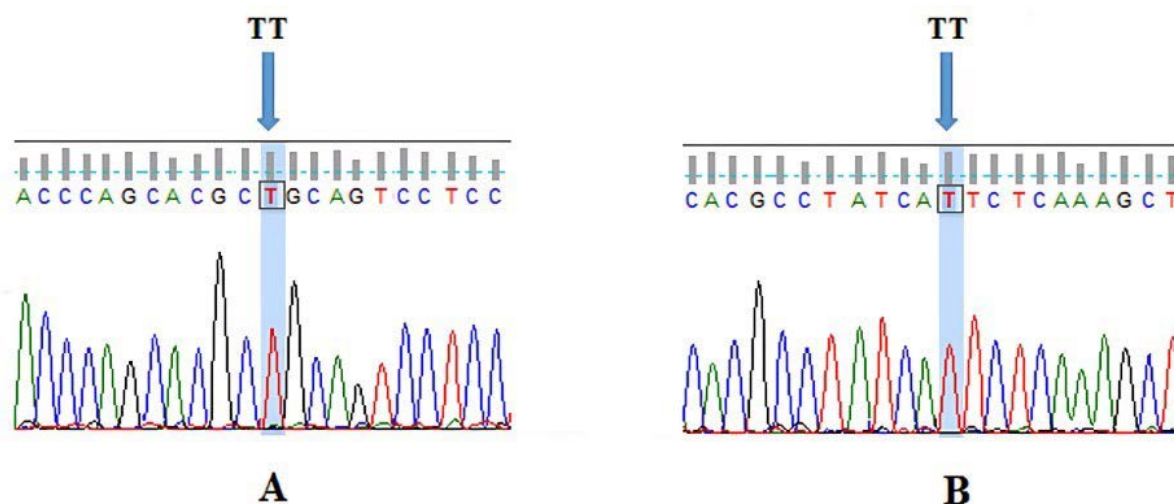


Figure 3. Sequencing of the -1306 C/T MMP-2 (A) and -1562 C/T MMP-9 (B) Polymorphisms in Individuals With the TT Genotype

were 62.1%, 26.2%, and 11.6%, respectively. Additionally, the frequency of C and T alleles was 56.8% and 43.1% in the first group, 58.1% and 41.8% in the second group, and 75.2% and 24.7% in the control group, respectively. The results of the chi-square test revealed a significant relationship between the frequency of the TT genotype and gastritis with *H. pylori* infection (OR=3.02, CI=1.33–6.85, $\chi^2=11.18$, $P=0.001$) and gastritis uninfected with *H. pylori* (OR=3.48, CI=1.64–7.40, $\chi^2=7.38$, $P=0.007$) in the studied groups. According to the obtained chi-square value, a significant difference was observed in the frequency of the T allele between the first group (OR=2.31, CI=1.54–3.44, $\chi^2=17.24$, $P=0.001$), the second group (OR=2.18, CI=1.42–3.34, $\chi^2=13.23$, $P=0.001$), and the control group. The obtained OR indicated that the T allele increased the risk of developing gastritis and *H. pylori* infection by 2.31 times (Table 3).

Statistical results demonstrated that the frequency of the TT genotype and T allele in the functional polymorphism of the MMP-2 and MMP-9 genes was higher in patients with gastritis infected with *H. pylori* (group 1) than in individuals with gastritis without infection (group 2). Additionally, another intergroup analysis was performed, comparing the total number of patients in the first and second groups (regardless of infection) with the control group in terms of genotypic and allelic frequencies. Based on the results of the chi-square test, there was a significant association between the frequency of the TT genotype (OR=2.47, CI=1.27–4.81, $\chi^2=9.50$, $P=0.002$) and that of

the T allele (OR=1.61, CI=1.23–2.11, $\chi^2=13.58$, $P=0.001$) in polymorphism -1306C/T in the studied groups. In polymorphism -1562C/T, a significant association was observed between the frequencies of the TT genotype (OR=2.41, CI=1.38–4.23, $\chi^2=11.79$, $P=0.001$) and the T allele (OR=1.79, CI=1.36–2.35, $\chi^2=19.43$, $P=0.001$) between the patient and control groups (Table 3). Similarly, genotypic and allelic frequencies between the first and second groups were examined, and the results demonstrated no significant relationship in the frequency of the TT genotype and T allele in polymorphism -1306C/T between the two groups. In polymorphism -1562C/T, the results showed that there was no statistically significant difference in the frequency of the TT genotype and T allele in both groups (Table 4). Overall, *H. pylori* infection did not affect the occurrence of -1306C/T and -1562C/T polymorphisms in the studied population.

Discussion

This study investigated the association between the promoter region polymorphisms of the MMP-2 and MMP-9 genes with gastritis and *H. pylori* infection in the Northern Iranian population. The results confirmed a significant relationship between the two functional polymorphisms in the MMP-2 and MMP-9 promoters (genotypic and allelic frequencies) with gastritis and *H. pylori* infection in the population under investigation. The statistically significant difference in the frequency of TT genotypes and T alleles at two loci between the

Table 3. Genotypic and Allelic Frequency Distribution of MMP-2 -1306C/T and MMP-9 -1562C/T Gene Polymorphisms in the Three Studied Groups

SNP	Genotype and allele	Group1 n (%)	OR (CI)	P-Value	Group2 n (%)	OR (CI)	P-Value	Group3 n (%)	OR (CI)	P-Value
MMP-2 rs243865	CC	49 (37.1)	-	-	32 (32.6)	-	-	57 (55.3)	-	-
	CT	60 (45.4)	1.83 (1.05-3.20)	0.032	49 (50)	2.29(1.25-4.20)	0.007	38 (36.9)	1.59 (1.13-2.24)	0.006
	TT	23 (17.4)	2.08 (1.11-3.88)	0.006	17 (17.3)	2.00(1.10-3.61)	0.004	8 (7.7)	2.47 (1.27-4.81)	0.002
	CT+TT	83 (62.8)	2.09 (1.24-3.54)	0.005	66 (67.3)	2.55(1.44-4.53)	0.001	46 (26.2)	1.77 (1.28-2.44)	0.000
	C	158 (59.8)	-	-	113 (57.6)	-	-	152 (74.7)	-	-
MMP-9 rs3918242	T	106 (40.1)	1.88 (1.27-2.80)	0.002	83 (22.3)	2.06(1.35-3.14)	0.001	54 (25.2)	1.61 (1.23-2.11)	0.000
	CC	52 (39.4)	-	-	37 (37.7)	-	-	64 (62.1)	-	-
	CT	46 (34.8)	2.09 (1.15-3.82)	0.015	40 (40.8)	2.56(1.35-4.83)	0.003	27 (26.2)	1.65 (1.16-2.35)	0.002
	TT	34 (25.7)	3.48 (1.64-7.40)	0.001	21 (21.4)	3.02(1.33-6.85)	0.007	12 (11.6)	2.41 (1.38-4.23)	0.001
	CT+TT	80 (60.6)	2.52 (1.48-2.28)	0.001	61 (62.2)	2.70(1.53-4.78)	0.001	39 (37.8)	1.93 (1.48-2.69)	0.000
	C	150 (56.8)	-	-	114 (58.1)	-	-	155 (75.2)	-	-
	T	114 (43.1)	2.31 (1.54-3.44)	0.000	82 (41.8)	2.18(1.42-3.34)	0.000	51 (24.7)	1.79 (1.36-2.35)	0.000

Note. SNP: Single-nucleotide polymorphism; *H. pylori*: *Helicobacter pylori*; OR: Odds ratio; CI: Confidence interval. Group 1: Patients with *H. pylori* infection; Group 2: Patients uninfected with *H. pylori*; Group 3: Normal (control).

Table 4. Genotypic and Allelic Frequency Distribution of MMP-2 -1306C/T and MMP-9 -1562C/T Gene Polymorphisms in the First and Second Groups

SNP	Genotype and Allele	Group 1 n (%)	Group 2 n (%)	OR (CI)	P-Value
MMP-2 rs243865	CC	49 (37.1)	32 (32.6)	-	-
	CT	60 (45.4)	49 (50)	1.25 (0.69-2.24)	0.45
	TT	23 (17.4)	17 (17.3)	1.13 (0.52-2.44)	0.75
	CT+TT	83 (62.8)	66 (67.3)	1.21 (0.70-2.11)	0.48
	C	158 (59.8)	113 (57.6)	-	-
MMP-9 rs3918242	T	106 (40.1)	83 (22.3)	1.09 (0.75-1.59)	0.63
	CC	52 (39.4)	37 (37.7)	-	-
	CT	46 (34.8)	40 (40.8)	1.22 (0.67-2.22)	0.51
	TT	34 (25.7)	21 (21.4)	0.86 (0.43-1.72)	0.68
	CT+TT	80 (60.6)	61 (62.2)	1.07 (0.62-1.83)	0.80
	C	150 (56.8)	114 (58.1)	-	-
	T	114 (43.1)	82 (41.8)	0.94 (0.65-1.37)	0.77

Note. SNP: Single-nucleotide polymorphism; *H. pylori*: *Helicobacter pylori*; OR: Odds ratio; CI: Confidence interval. Group 1: Patients with *H. pylori* infection; Group 2: Patients uninfected with *H. pylori*.

patient and control groups suggested that the presence of these alleles at the above two loci could be a risk factor for disease progression and infection with *H. pylori*. Additionally, CC genotypes in the promoter region of two genes are perceived as a protective factor against disease, reducing the risk of *H. pylori* infection. Gastritis, a common issue in communities, is characterized by the inflammation of the stomach's mucous membrane. In addition, *H. pylori* is widely acknowledged as the primary contributor to the development of gastritis, peptic ulcer, and gastric cancer. *H. pylori* gastritis, and especially the subsequent atrophic gastritis, increases the risk of stomach cancer for several reasons (16). Damage to the gastric mucosa is associated with the degradation of the ECM, in which MMPs play a pivotal role. It should be noted that MMPs are involved in various biological processes, such as ECM remodeling and inflammation. Likewise, studies have shown that MMPs substantially contribute to various gastrointestinal pathologies, including gastric diseases (17). Genetic variation in MMP genes may play a crucial role as a genetic risk factor in the development and progression of chronic *H. pylori* infection. Research indicated that *H. pylori* infection correlates with the increased expression of MMP-2 and MMP-9 in gastric mucosal cells (18). A meta-analysis study revealed that the rs3918242 polymorphism in the MMP-9 promoter could potentially be linked to an increased susceptibility to gastric cancer (19). Similarly, Fu et al reported that the presence of the T allele at the MMP-9 locus in the rs3918242 promoter may be associated with a higher risk of gastric cancer and metastasis (20). Furthermore, Karayiannis et al concluded that colonization by *H. pylori* was related to the increased expression of MMP-3 and MMP-9; inhibiting these enzymes could potentially have a protective effect against gastric carcinogenesis and metastasis (21). Moreover, Kong et al demonstrated that MMP-9 mediates inflammatory responses induced by *H. pylori* infection. They also found that *H. pylori* stimulation can increase the proliferation and migration of gastric cancer cells, but these effects are sharply reduced by inhibiting MMP-9. Their research suggests that MMP-9 plays a role in the progression of gastric cancer and that targeting MMP-9 may be a therapeutic strategy to combat the development of gastric cancer caused by *H. pylori* (22). Based on the finding of one study, MMP-9 gene promoter polymorphisms were associated with *H. pylori*-infected gastric ulcers but not with *H. pylori*-negative gastric ulcers (23). Zhang et al reported that single-nucleotide variations at the -1306C/T locus in the MMP-2 promoter may be related to susceptibility to gastric cancer. They concluded that the presence of the T allele at the -1306C/T locus could be considered a potential risk factor for gastric cancer (24). A review study also revealed that patients with chronic gastritis associated with *H. pylori* infection show increased expression of MMP-2 (25). The results of researchers' studies on various diseases revealed that single-nucleotide changes in the promoters of the MMP-2 and MMP-9 genes are linked to different human diseases.

Further, Gao et al found that polymorphisms in the MMP-2 gene promoter are linked to several human cancers, including prostate, lung, stomach, and bladder cancer. They also noted that these polymorphisms are related to MMP-2 expression, clinical characteristics of cancer patients, and tumor susceptibility. Therefore, it could be introduced as a new diagnostic and prognostic biomarker for a range of human diseases (26). Likewise, Reddy et al reported that MMP-2 could be an essential factor in cancer progression in humans (27). The results of various studies likely indicated the different effects of genetic and environmental factors on the disease. This study focused on investigating a functional polymorphism in the promoter region of the MMP-2 and MMP-9 genes. Given the limitations of our study (e.g., the small population, size, and low number of samples) and the multifactorial nature of gastritis disease and its complex pathogenesis, it is necessary to evaluate other polymorphisms in the MMP gene family in larger populations across different regions. More precisely, identifying the effect of these functional polymorphisms will further our understanding of the genetic pathways of the disease. Therefore, manipulating the expression or function of MMP-2 and MMP-9 could be a potential therapeutic strategy for various diseases, including gastrointestinal diseases.

Conclusion

In this study, a significant association was observed between functional polymorphisms in the MMP-2 and MMP-9 gene promoters. The presence of the T allele at polymorphic sites -1306C/T and -1562 C/T seems to be associated with susceptibility to gastritis and *H. pylori* infection in the studied population. Considering that gastritis is a multifactorial disease, it is essential to investigate other polymorphisms of these two genes and other genes of the MMP family, as well as environmental factors that are effective in the development of the disease, in order to simultaneously evaluate the impact of different factors.

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 Formal Analysis: Rasoul Zahmatkesh Roodsari
 Funding Acquisition: Mrjan Soltani
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Competing Interests

The authors declare they have no competing interests.

Ethical Approval

The present study was approved by the Ethics Committee of the Faculty of Medical Sciences, Islamic Azad University, Tonekabon Branch (IR.IAU.TON.REC.1402.007).

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