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Interleukin-8 genetic polymorphism and its relation to *Helicobacter pylori* infection and *Helicobacter pylori*-associated gastric diseases

Ibrahim A. Amin[1], Mona A. Hassan[2], Sherein G. Elgendy[2], Ahmed S. Abdelmohsen[3], Mamdouh Y. Ali[1], and Bahaa-Eldin A. Abdel-Raady[1]

[1]Department of Microbiology & Immunology, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.
[2]Department of Medical Microbiology & Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt.
[3]Department of Tropical Medicine & Gastroenterology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Corresponding author: Ibrahim A. Amin, Department of Microbiology & Immunology, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.
Email: malkibrahim2219872991991@gmail.com.

Abstract

Helicobacter pylori (H. pylori) infection has a variety of clinical outcomes, and host genetic factors play an important role in this process. Cytokines are important factors in mediating and controlling the inflammatory process during *H. pylori* infection. Interleukin-8 (IL-8) plays a critical role in the epithelial cell response to *H. pylori* infection and the development of *H. pylori*-related gastric disorders. The IL-8 gene has an A/T base pair polymorphism in the promoter region (-251), which has been linked to an increase in interleukin production by gastric epithelial cells. In this context, the goal of our study was to determine the polymorphism in the IL-8 gene and its relation to *H. pylori* infection and *H. pylori*-associated gastric diseases. Gastric biopsy specimens were collected from 44 patients with *H. pylori* infection and 29 patients without *H. pylori* infection. The rapid urease test and detection of the glmM gene were used to diagnose *H. pylori* infection. Polymerase chain reaction-restriction fragment length polymorphism was used to identify the polymorphism in the Il-8 gene (at position-251). The presence of the A/A and T/A genotypes of the IL-8 gene was found to be significantly associated with susceptibility to *H. pylori* infection (p = 0.012 and p = 0.004, respectively). Also, the IL-8 A allele was significantly associated with *H. pylori* infection in our study (p = 0.002). We did not find a significant association between IL-8 gene polymorphism and a higher risk of gastritis and peptic ulcer disease. In conclusion, IL-8 gene polymorphism at -251 position was significantly associated with *H. pylori* infection.

Keywords: Genetic polymorphism, Interleukin-8 gene, *H. pylori*, gastric disorder.

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Introduction

Since its first isolation by John Robin Warren and Barry J. Marshall in 1982, *Helicobacter pylori (H. pylori) has* become an area of interest for many specialists like microbiologists, oral health professionals pathologists, and

gastroenterologists.[1] This spiral bacterium colonizes the stomachs of about 50% of the world's population,[2] causing different clinical outcomes from normal mucosa to gastritis, peptic ulcer disease (PUD), and cancer.[3] Variation in the clinical outcome in *H. pylori* colonized patients contributes to different factors, including bacterial genetic makeup, host genetic factors, and environmental factors.[4] The most important event during *H. pylori* infection is the production of an inflammatory response in the gastric mucosa,[5] which has an important role in the development of gastric pathologies.[6] The inflammatory response produced in the gastric mucosa during *H. pylori* infection is produced by the action of pro-inflammatory cytokines (e.g. interleukin (IL)-1β, IL-2, IL-6, IL-8 and tumor necrosis factor (TNF)-α).[7]

IL-8, also known as CXCL8, belongs to a group of cytokines known as chemokines.[8] This class of cytokines is pro-inflammatory and their main function is the activation and migration of neutrophils from peripheral blood to the site of inflammation.[9] IL-8 was initially identified and isolated as a powerful chemoattractant factor for neutrophils and interact with two chemokines receptors (CXCR1 and CXCR2).[10] IL-8 was shown to be the most noticeably upregulated gene in whole genome analysis of the epithelial response to *H. pylori* infection.[11] As an early response to *H. pylori* infection, gastric epithelial cells produce IL-8, which induces mucosal damage by stimulating the release of reactive oxygen species.[12]. Thus, IL-8 plays a critical role in the epithelial cell response to *H. pylori* infection and the development of *H. pylori*-related gastric disorders.[13] It was reported that the IL-8 gene is one of the most significant prospective host genes for predicting the outcome of *H. pylori* infection.[14]

By using somatic cell hybridization and in situ hybridization, the human IL-8 gene was identified as being located on the 4q12–q21 gene cluster. Its length is about 5.2 kb and it contains 10 exons.[15] IL-8 gene has an A/U-rich element in its 3' untranslated region, which makes it extremely unstable in certain circumstances.[16] The IL-8 gene has a single nucleotide polymorphism (SNP) of an A/T type in the promotor region (-251) (rs4073), which is associated with increased IL-8 production by gastric epithelial cells.[17] This SNP of IL-8 has been linked to the development of various inflammatory disorders and diseases, including gastric cancer.[18]

The present study aimed to determine the frequency of host IL-8 gene polymorphism in patients with gastroduodenal disorders in Assiut University Hospitals, to explore the effect of this polymorphism on the risk of *H. pylori* infection, and to clarify the association between this polymorphism and normal gastric mucosa, gastritis, and PUD.

Patients and Methods

Patients

The present study included 73 patients with gastroduodenal disorders who underwent upper gastric endoscopy at the Endoscopy Unit of Assiut University Hospitals. A complete history was recorded for each patient, including name, age, sex, and any prior administration of antibiotics. Inclusion criteria included all patients with gastrointestinal complaints, particularly those with dyspeptic symptoms (such as nausea, vomiting, and epigastric pain).[19] Patients who had undergone partial or total gastrectomy, those with a previous administration of *H. pylori* therapy,[20] and those who had received antimicrobials within 4 weeks or stomach acid-reducing drugs within the last 2 weeks were all excluded from the study.[21]

Diagnosis of *H.pylori* infection was carried out by the rapid urease test and detection of the *glmM* gene by polymerase chain reaction (PCR). The diagnosis of gastroduodenal disorders was done by endoscopic examination.

Collection of Gastric biopsies

Two antral biopsy samples were obtained from each patient. The first biopsy sample was used for the rapid urease test, while the second sample was put in physiological saline and preserved at -70 °C until used for PCR and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

Rapid urease test

Immediately after collection, the first biopsy specimen was used for the detection of *H. pylori* urease enzyme by using the rapid urease test stripts (Association of Medicine and Analytics Co Ltd, Russia), according to the manufactutrer's instrusctions.[22] The positive result was obtained when the color on the strip around the biopsy changed from yellow to red.

DNA extraction and PCR

The Genejet DNA purification kits (Thermo Fisher Scientific Inc, USA) were used to extract DNA from gastric biopsy samples as per the manufacturer's instructions.[23] Diagnosis of *H. pylori* infection by PCR was carried out by using the glmM Forward primer (5'-AAGCTTTTAGG GGTTTAGGGTTT-3') (Willowfort, UK) and glmM Reverce primer (5'-AA GCTTACT TTCTA ACACTAA CGC-3') (Willowfort, UK), which amplifies a 294-bp fragment that corresponds to the *glmM* gene. The volume of the PCR reaction mixture was 25 µl containing 1 µl of each of the forward and reverse primers, 5µl template DNA, and 10 µl of 2X of MyTaq™ Mix (Bioline, UK). The reaction was carried out in a thermal cycler (Biometra, Germany) at the following cycling conditions: 94 °C for 5 minutes of initial denaturation; 40 cycles each of 94 °C for 1 minute; 55 °C for 1 minute; and 72 °C for 1 minute; followed by final extension at 72 °C for 10 minutes.[24]

Analysis of IL-8 Polymorphism

PCR-RFLP was used to analyze the IL-8 polymorphism at position 251. The sequence of the PCR primers was as follows: forward primer, (5'-TTCTAACACCTGCCACTCTAG-3') and reverse primer, (5'-CTGAAGCTCACAATTTGGTG-3').[25] The total volume of the PCR reaction mixture was 20 µl containing 1µl of each of the forward and reverse primers, 5 µl of template DNA, and 10 µl of 2X of Master Mix. The DNA was first denatured at 94 °C for 4 minutes, then subjected to 40 cycles each of heating at 94 C for 30 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds, followed by a final extension at 72 °C for 10 minutes.[25] To investigate the IL-8 251 T > A polymorphism, PCR products were digested overnight at 37 °C employing 5 units of *MfeI* restriction enzyme (New England Biolabs, Inc., Bevely, MA).[25] The results of the digestion were then run on 5% agarose gel electrophoresis stained with ethidium bromide. The IL-8 (-251) genotypes were coded in the following way: T/T has one 108-bp band; T/A has three 108-bp, 76-bp, and 32-bp bands; and A/A has two 76-bp and 32-bp bands.[25]

Statistical Analysis

All statistical analyses, which were based on the generation and classification of variables, were performed using the Statistical Program for the Social Sciences (SPSS) version 26.0 (IBM, Inc., Chicago, USA). The differences in age between the different groups were assessed using an analysis of variance. The chi-square (x^2) test was used to investigate differences between categorical variables. The association was estimated by calculating odds ratios (ORs) and their 95 % confidence intervals (CIs) using multivariate logistic regression. A p-value < 0.05 was considered statistically significant.

Results

A total of 73 patients undergoing upper gastrointestinal endoscopy were enrolled in this work. Of these, 35 (48%) were males, and 38 (52. %) females. The mean age of the studied patients was 42.26 ± 13.45 years. The minimum age was 18.0 years and the maximum age was 74.0 years. Patients were considered *H. pylori* positive when positive results were obtained by PCR (Figure1) and/or the rapid urease test and considered negative when the results of the two tests were negative.

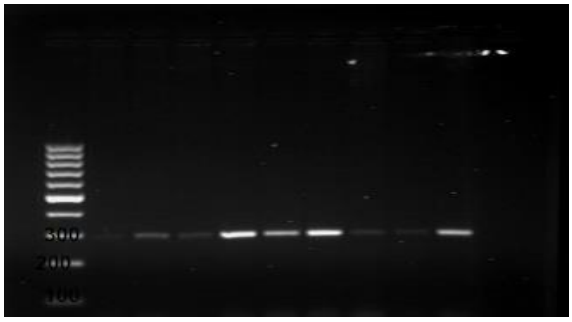

Figure 1. A photmicroograph of 1.5% agarose gel showing the product of a representative PCR experiment for detection of *H. pylori* glmM gene (297-bp). lane 1=100 bp DNA ladder.

A total of 44 patients (60.3%) were diagnosed as having *H. pylori* infection, and 29 patients (39.7%) negative for *H. pylori* infection. Patients were then divided into two groups according to *H. pylori* infection. Age and sex were not different between the two groups. Significant differences in endoscopic findings between the two groups were obtained, which indicated that *H. pylori* infection was significantly associated with gastritis and PUD (Table 1).

Using the PCR-RFLP technique (Figure 2), the IL-8 gene was genotyped at position -251. As shown in Figure 3, the most common genotype was TA in 33 patients (45%), followed by TT in 26 patients (36%) and the AA genotype in 14 patients (19%).

Table 1. Baseline demographic characteristics and endoscopic findings of two study groups.

Studied parameter	*H. Pylori infection*		*p*-value
No. (%)	Positive 44 (60.3%)	Negative 29 (39.7%)	
Age, years	41.30±13.187	43.72±13.94	NS
Age (min-max)	18-67	18-74	
Sex			
Male	19 (43.2%)	16 (55.2%)	NS
Female	25 (56.8%)	13 (44.8%)	
Endoscopic findings			
Gastritis	24 (54.5%)	8 (27.6%)	<0.001
Peptic ulcer	14 (31.8%)	3 (10.3%)	
Normal gastric mucosa	6 (13.6%)	18 (62.1%)	

Data presented as mean ± SD or n of patients (%); Age was compared using analysis of variance; χ2-test was used to compare categorical variables; $P > 0.05$ is not significant (NS).

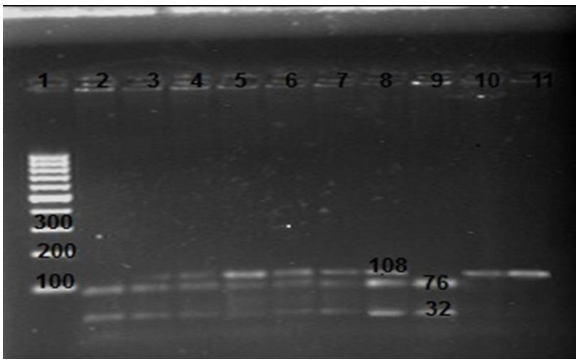

Figure 2. PCR-RFLP analysis of IL-8 (-251) polymorphism by *MfeI* enzyme after gel electrophoresis. (lane 1 = ladder marker). Lane 3, 4, 5, 6, 7, 8 indicate A/T genotype (3 bands of 108 bp, 76 bp and 32 bp); lane 2 & 9 indicate A/A genotype (2 bands of 76 bp & 32 bp) & lane 10 & 11 indicate T/T genotype (single band of 108 bp).

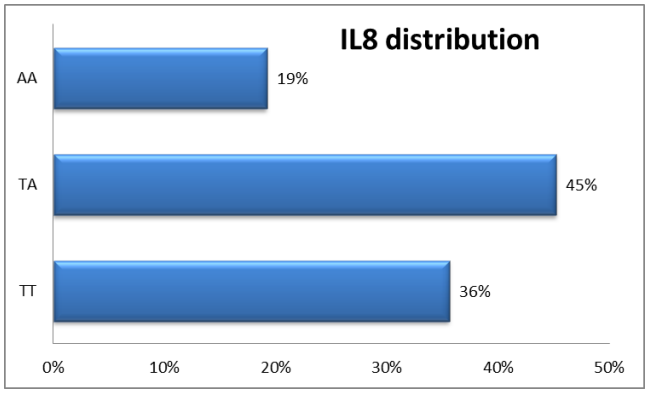

Figure 3. Frequency of IL-8 genotypes in the 73 patients with dyspepsia.

The genotypic and allelic frequency of host IL-8 gene polymorphism at the -251 position in *H. pylori*-positive cases and *H. pylori*-negative cases are shown in Table 2. The presence of the A/A and T/A genotypes of the IL-8 gene was found to be significantly associated with susceptibility to *H. pylori* infection (p = 0.012 and p = 0.004, respectively). Also, the IL-8 A allele was significantly associated with *H. pylori* infection in our study (p = 0.002).

Table 2. The frequency of IL-8 gene polymorphism in *H. pylori*-positive and *H. pylori*-negative patients.

	H. Pylori positive		*H. Pylori* negative		Odds Ratio (95% CI)	*p*-value
	NO	%	NO	%		
IL-8 genotypes						
TT	9	20.50	17	58.60	1	Ref
TA	24	54.50	9	31.10	5.03 (1.654-15.336)	0.004
AA	11	25.00	3	10.30	6.92 (1.529-31.377)	0.012
Total	44	100	29	100		
IL-8 alleles						
T	42	47.70	43	74.13	1	Ref
A	46	52.30	15	25.87	3.14 (1.526-6.459)	0.002
Total	88	100	58	100		

p-value was calculated by multivariate logistic regression. A *p*-value < 0.05 was considered statistically significant. 95% CI, 95% confidence interval; IL-8, interleukin-8.

The frequency of IL-8 gene polymorphism in *H. pylori*-positive and *H. pylori*-negative patients with normal gastric mucosa, gastritis, and PUD is shown in Table 3. The results shown in this table revealed that there was no significant difference in genotypic and allelic frequency of the IL-8 gene regarding *H. pylori* infection in gastritis and PUD patients.

Table 3. The frequency of IL-8 gene polymorphism in *H. pylori*-positive and *H. pylori*-negative patients with normal gastric mucosa, gastritis, and PUD.

	Gastritis (n=32)			*p* value
	H.pylor (+ve) n (%)	*H.pylori* (ve) n (%)	Odds Ratio (95% CI)	
IL-8 genotypes				
TT	5 (20.38)	5 (62.50)	1	Ref
TA	13 (54.20)	2 (25.00)	6.50 (0.937 45.106)	NS
AA	6 (25.42)	1(12.50)	6.00 (0.516- 69.754)	NS
Total	24 (100)	8 (100)		
IL-8 alleles				
T	23 (48.00)	12 (75.00)	1	Ref
A	25 (52.00)	4 (25.00)	3.261(0.920-11.558)	NS
Total	48(100)	16(100)		
	Peptic ulcer (n=17)			*p* value
	H.pylori (+ve) n (%)	*H.pylori* (ve) n (%)	Odds Ratio (95% CI)	
IL-8 genotypes				
TT	3 (21.43)	2 (66.67)	1	Ref
TA	7 (50.00)	1 (33.33)	4.667 (0.297-73.384)	NS
AA	4 (28.57)	0 (0.00)	-	-
Total	14(100)	3(100)		
IL-8 alleles				
T	13 (46.42)	5 (83.33)	1	Ref
A	15 (53.58)	1 (16.67)	5.76 (0.595-55.947)	0.131
Total	28 (100)	6 (100)		
	Normal gastric mucosa (n=24)			*p* value
	H.pylori (+ve) n (%)	*H.pylori* (-ve) n (%)	Odds Ratio (95% CI)	
IL-8 genotypes				
TT	1 (16.66)	10 (55.55)	1	Ref
TA	4 (66.68)	6 (33.33)	6.66 (0.597-74.506)	NS
AA	1 (16.66)	2 (11.12)	5.00 (0.212- 117. 894)	NS
Total	6 (100)	18 (100)		
IL-8 alleles				
T	6 (50.00)	26 (72.22)	1	Ref
A	6 (50.00)	10 (27.78)	2.60 (0.677-9.992)	NS
Total	12 (100)	36 (100)		

p-value was calculated by multivariate logistic regression. $p > 0.05$ is not significant (NS). CI, 95% confidence interval; IL-8, interleukin-8.

Discussion

The clinical outcome of *H. pylori* infection is not the same in all individuals and depends on how the host interacts with this pathogen. Cytokine gene polymorphism affects gene expression and susceptibility to infectious diseases.[26] The current study aimed to explore the relation between host IL-8 gene polymorphism at -251 position and the risk of *H. pylori* infection. In our study, a significant association was found between the presence of the A/A, T/A genotypes, and A allele of the IL-8 gene and susceptibility to *H. pylori* infection (p = 0.012, p = 0.004, p=0.002, respectively). This indicates that the T/A and A/A genotypes may be risk factors for *H. pylori* infection, but the T/T genotype may operate as a protective factor against *H. pylori* infection. In the same manner, Saha et al., 2016,[27] conducted a study on Bangladeshi patients and they found a significant association between host IL-8 genotypes (T/T, T/A, A/A, and A carrier) and the presence of *H. pylori* infection. Also, Ramis et al., 2017,[28] reported that the presence of the A allele at position -251 of the IL-8 gene was significantly associated with *H. pylori* infection. Similar results were also obtained in Brazil[29]. Thye et al., 2003,[30] carried out a genome-wide linkage study to find the host genetic factors influencing the likelihood of *H. pylori* infection and found a potential association between the host factors and chromosomes 4 and 6. Their findings may support the hypothesis that the IL-8 gene polymorphism is associated with *H. pylori* infection because the human IL-8 gene is found on chromosome 4 (4q13-q21). In contrast, Fabris et al., 2011,[31] in Brazil, and Farshad et al., 2010,[32] in Iran found that IL-8-251 T/A polymorphism had no impact on *H. pylori* susceptibility.

In a meta-analysis, Xue et al., 2012,[33] found that the IL-8-251AA genotype is not associated with susceptibility to *H. pylori* infection. Zhao et al., 2013,[34] conducted a study to investigate the relationship between *H. pylori* infection and the host genetic makeup of Indonesian healthy populations. In their study, there was no statistical significance between *H. pylori* infection and polymorphisms in IL-8, IL-4, IL-1b, CD14, TNF-α, and tyrosine-protein phosphatase nonreceptor type 11 (PTPN11). These conflicting results may be attributed to the genetic makeup of *H. pylori* in different geographical areas and the variations in the *H. pylori* detection techniques employed in these studies.

In our study, we observed no significant association between IL-8 polymorphism and *H. pylori* infection in gastritis patients. In agreement with our finding, Cheng et al., 2010,[35] found no association between polymorphisms of IL8-251 T/A with an increased risk of gastritis in Thai patients. Also, Ramis et al., 2017,[28] and Kamali-Servestani et al., 2006,[14] found no significant association among gastritis patients. Hofner et al., 2007,[36] reported a significant association of IL-8 T/A genotype with the risk of developing gastritis in *H. pylori*-infected patients, which contrasts with our finding. Also, Saha et al., 2016,[27] showed that the IL-8 A carrier was significantly associated with *H. pylori*-infected gastritis patients. These conflicting findings could be attributed to genetic and ethnic variations within populations as well as variations in research methodologies, sample sizes, *H. pylori* detection methods, and dietary practises.

The current study observed no correlation between IL-8 polymorphism and *H. pylori* infection in PUD patients. Our result is in agreement with that obtained by Kamali-Servestani et al., 2006,[14] and Farshad et al., 2010.[32] The most thorough examination of the association between the IL-8 gene -251 T/A polymorphism and PUD risk was provided by a meta-analysis of eight studies, which included 1262 PUD patients and 1843 controls. The overall findings of this study revealed that the IL-8 gene -251 T/A polymorphism did not affect on the development of PUD.[37] On the other hand, our finding conflicts with that obtained by Saha et al., 2016,[27] who found that individuals with *H. pylori* infection who also carried the A allele for the IL-8 gene at position -251 were more likely to develop peptic ulcer disease. However, several studies found the opposite of our result.[28,36,38] Diverse study designs, sample sizes, population demographics, lifestyle choices, and environmental factors can all

contribute to the disparities in results between different studies.

The present study has two limitations. No sequencing was performed for confirmation of IL-8 genotypes detected by PCR-RFLP analysis due to limitations in resources and budget. The second limitation was the relatively limited number of patients who participated in this study, diagnosed with gastritis and PUD. In conclusion, this study demonstrated that IL-8 -251 T/A polymorphism may be associated with an increased risk of *H. pylori* infection.

Author Contributions

All authors made a significant contribution to this work, whether that is in the conception, study design, acquisition of data, analysis, and interpretation, or in drafting, revising, or reviewing the article and gave final approval of the version to be published.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Assiut University (Approval dated: May 2022).

Informed consent

Written informed consents were taken from each study participant before being included in the study.

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