Association of Myeloperoxidase -463 G/A Polymorphism with Clinical Outcome of *Helicobacter Pylori* infection in Iranian Patients with Gastrointestinal Diseases

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ABSTRACT

Background: Polymorphisms in the immune related genes are important in the clinical outcome of *Helicobacter pylori* infection. Myeloperoxidase -463 G/A polymorphism has been shown to reduce enzyme expression and activity. **Objective:** the aim of the present study is to investigate the association of myeloperoxidase G-463A polymorphism with clinical outcome of *Helicobacter pylori* infection. **Methods:** two hundred and eighty five patients with positive culture of *Helicobacter pylori* from their gastric biopsies are included in this study. Human leukocyte DNA was extracted using salting out method and myeloperoxidase G-463A polymorphism was investigated by PCR-RFLP. All clinicopathological data were collected from individual records. **Results:** When the patients were categorized according to the high (GG) and low + intermediate (AG+AA) genotypes of myeloperoxidase producers, there was a significant association between myeloperoxidase G-463A genotypes and clinical outcome of *Helicobacter pylori* infection (p=0.006). In search for combined effect of *cag*A status and myeloperoxidase genotypes on clinical presentations, only in *cag*A$^-$ *Helicobacter pylori* infected patients a significant association between myeloperoxidase genotypes and clinical outcome was found (p=0.0001). Also this association was found only in patients infected with *vac*A s1m1 genotype (p=0.008). **Conclusions:** Our findings suggest that the myeloperoxidase G-463A polymorphism is a host genetic factor which determines the clinical outcome of *Helicobacter pylori* infection. Moreover, the combination of host and bacterial genetics could provide a better understanding of clinical outcome after infection with *Helicobacter pylori*.

Keywords: *Helicobacter pylori*, Myeloperoxidase, Polymorphism

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INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is the major cause of chronic superficial gastritis in humans and an important etiological factor in the pathogenesis of peptic ulcers (PUD) and some forms of gastric cancer (1). However, most people who harbor *H. pylori* are asymptomatic, and only a few patients infected with this bacterium develop either peptic ulcer or gastric cancer (2). The variability of clinical manifestations is associated with several factors such as specific organism virulence factors, host immunological factors, and environmental milieus (3).

In studies conducted in several countries, the vacuolating cytotoxin (VacA) and cytotoxin-associated protein (CagA) are considered as the two major virulence markers usually associated with *H. pylori* pathogenicity (4-9). Recently, attention has been focused on the involvement of host factors that determine susceptibility to *H. pylori* associated diseases such as human leukocyte antigen and genetic polymorphisms in the cytokine genes (10-12). It is well known that *H. pylori* infection is characterized by extensive infiltration of neutrophils. Neutrophils may generate superoxide anions (O_2^-), hydroxyl radicals (OH^o) and non radical oxidants such as hypochlorous acid in order to kill bacteria (13-15). Myeloperoxidase, a key enzyme in neutrophils, is involved in the formation of hypochlorous acid from H_2O_2 in the presence of chloride ions. HOCl is a potent oxidant known to have several cytotoxic effects on bacterial cells (14). Additionally, activated neutrophils and monocytes can also generate cytotoxic chloramines, tyroxyl radicals, and OH^o via a myeloperoxidase-dependent pathway (13-15). Interestingly, the host ability in myeloperoxidase production is partially determined by a few polymorphisms which are described in the myeloperoxidase gene. Among them, a functional G-463A polymorphism located in the promoter region of this gene affects its transcription. This polymorphism is believed to alter an SP1 transcription factor binding site (16). The presence of an A rather than a G at this site destroys the binding site for SP1 and decreases the expression of myeloperoxidase enzyme in the cell (16). The role of this polymorphism has been studied in many diseases and there is an association between this polymorphism and Alzheimer's disease (17), lung cancer (18), and some complications of chronic granulomatosis disease (19). Therefore, in the present study we investigated the association of myeloperoxidase G-463A polymorphism with clinical outcome in *H. pylori* infected patients and tried to find out if there is any interaction between *cag*A and *vac*A genotype of *H. pylori* with G-463A polymorphism in determining the outcome of the disease.

MATERIALS AND METHODS

Patients. *H. pylori* was isolated from biopsy specimens sampled from the antrum and the corpus of 264 patients (137 men, 127 women; median age, 49 years; age range, 15 to 86 years) in Shiraz University Hospitals, Iran, as described previously (20). The final diagnosis was gastritis in 200 patients, peptic ulcer (PUD) in 67 patients, and gastric cancer in 18 patients. Gastritis patients had chronic histological gastritis without peptic ulcer, gastric cancer, or esophageal disease. Gastric cancer patients had no other primary malignancies or inflammatory diseases. The present study was approved by the local ethics committee.

DNA Isolation and Myeloperoxidase (MPO) Genotyping. Genomic DNA was extracted from patients by salting out method. For determination of the G-463A exchange, a 350-bp DNA fragment was amplified using 5´-CGGTATAGGCACACAATGGTGAG-3´ as a forward primer and 5´-GCAATGGTTCAAGCGATTCTTC-3´ as a reverse primer as described (18). PCR conditions were 94˚C for 5 min followed by 35 cycles of 94˚C for 30s, 56˚C for 30s, and 72˚C for 30s. Seven microliters of PCR product was digested with 2 units of *Aci*I restriction enzyme (New England Biolab) in 2µL of 10X buffer and 5.7µL of water at 37˚C overnight. Fragments were separated using 2.5% agarose gel. Three possible genotypes are defined by 4 distinct banding patterns: 289 and 61 bp fragments for AA; 289, 161, 121 and 61 bp fragments for AG; and 161, 121 and 61 bp fragments for GG genotypes (Figure 1).

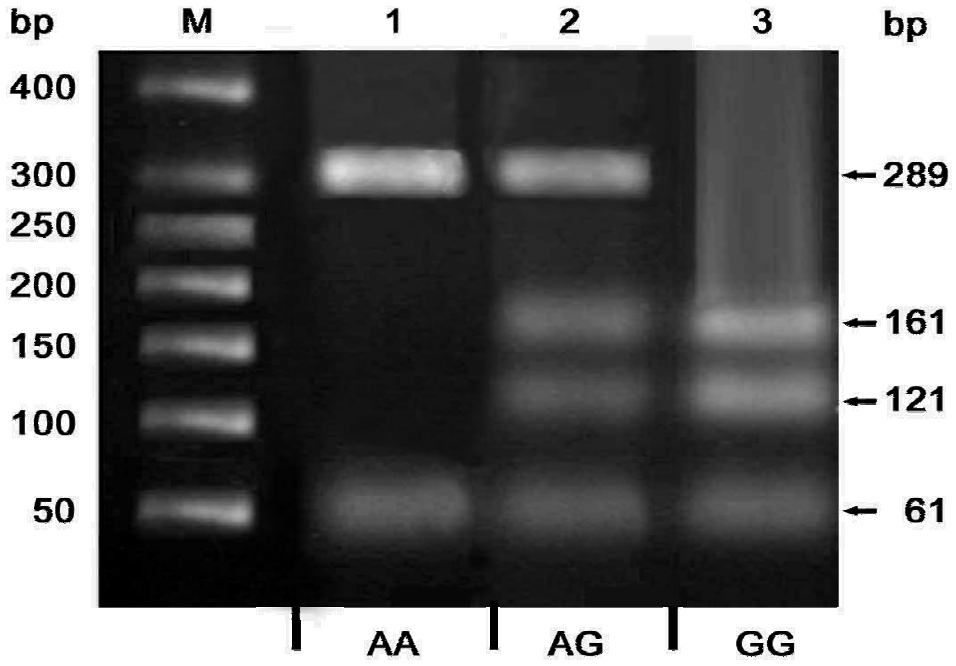

Figure 1. Electrophoresis on a 2.5% agarose gel of myeloperoxidase DNA fragments stained with ethidium bromide. Lane M: DNA size standard (100 bp ladder); Lane1: *Aci*I digestion of homozygous mutant (-463A/A); Lane 2: heterozygous mutant (-463G/A); Lane 3: homozygous wild type (-463G/G).

Statistical Analysis. Allele and genotype frequencies were calculated on patients by direct gene counting. Statistical analysis of the differences between groups was determined by chi-square test using EPI 2000 and SPSS software version 11.5. Findings were considered statistically significant at a P value < 0.05. Analysis for deviations from Hardy-Weinberg Equilibrium was performed based on the Pearson ($\chi 2$) test.

RESULTS

The distribution of myeloperoxidase genotypes in patients with different clinical presentations is demonstrated in Table 1. Frequency of myeloperoxidase G-463A polymorphism was significantly different among patients with different clinical manifestations (p=0.004). Interestingly, myeloperoxidase G-463A polymorphism did show a significant difference between peptic ulcer and gastritis patients (p=0.001). The reason for this difference is the higher frequency of GG genotypes in peptic ulcer patients (82.5%) compared to the gastritis ones (60.4%). In fact, when the genotypes were categorized as high myeloperoxidase producers (GG) and low + intermediate myeloperoxidase producers (AA+AG), the frequency of GG was significantly higher than AA+AG in peptic ulcer patients compared to the gastritis cases (p=0.002; OR=3.09, 95% CI=1.44–6.76). Moreover, if the combination of cagA status and myeloperoxidase genotypes was considered, only in patients infected with $cagA^-$ bacteria, the difference in GG and AA+AG distribution remained significant between peptic ulcer and gastritis patients (Table 2; p=0.016). Similarly, the difference in distribution of GG and AA+AG genotypes between peptic ulcer and gastritis patients was only significant in patients infected with s1m1 genotype of *H. pylori* (Table 2; p=0.008).

Table 1. Distribution of myeloperoxidase G-463A genotypes in patients with gastric diseases

Disease Genotype	Gastritis n (%)	Peptic ulcer n (%)	Gastric cancer n (%)	P value
AA	7(3.8)	4(6.3)	2(10.5)	0.37
AG	65(35.7)	7(11.1)	5(26.3)	0.001
GG	110(60.4)	52(82.5)	12(63.1)	0.006

Table 2. Distribution of myeloperoxidase genotypes and H. pylori virulence factors (CagA and VacA) in patients with gastritis (G) and peptic ulcer (PU)

	Myeloperoxidase				P value
	AA+AG		GG		
	G (%)	PU (%)	G (%)	PU (%)	
Cag+	52 (88)	7 (22)	97 (77)	30(23)	0.090
Cag-	24 (100)	0 (0)	27 (77)	8 (23)	0.0001
S1m1	23 (96)	1 (4)	26 (63)	15 (37)	0.008
S1m2	24 (80)	6 (20)	51 (76)	16 (24)	0.870
S2m2	22 (100)	0 (0)	36 (83)	7 (16)	0.080

DISCUSSION

H. pylori has become a world-wide infective agent ranging from 25% in developed countries to more than 80% in the developing world. Not all individuals infected with *H. pylori* develop gastric illnesses and this might be related to various bacterial and host genetic factors.

Besides the effects of cytokine gene polymorphisms on clinical outcome of *H. pylori* infection (20), Roe et al. reported the importance of myeloperoxidase polymorphism as a host factor in determination of the severity of gastric atrophy in *H. pylori*-colonized

gastric mucosa (21). They showed a strong association between the level of gastric atrophy and the wild myeloperoxidase GG genotype. Association of myeloperoxidase G-463A allele and the development of duodenal ulcer disease has also been reported (22). Our results suggest that myeloperoxidase genotype is associated with clinical outcome of *H. pylori* infection (p=0.004). In fact, the rate of low + intermediate expressing genotypes (AA + AG) of myeloperoxidase gene was higher in patients with gastric cancer (36%) than in patients with peptic ulcer disease (16.5%). Therefore, a lower genetic ability of the host in myeloperoxidase production could predispose the patients to gastric cancer by permitting bacterial survival. Supporting this hypothesis, Hsu et al. (22) reported that *H. pylori*-infected myeloperoxidase -463A carriers had higher scores of bacterial density. High bacterial loads may stimulate more gastrin release, which can lead to increased cell proliferation and inhibition of apoptosis through production of proinflammatory cytokines and reactive oxygen species (23). The results could be increased risk of gastric cancer in infected patients.

All strains of *H. pylori* have the gene encoding the toxin vacA, but its structure varies, especially in the mid-region which may be type m1 or m2 and the region encoding the signal sequence (type s1 or s2). The final structure is a mosaic, and our previous study showed that all combinations of the signal sequence and the mid-region types are found in our patients except s2/m1 (20). In vitro studies showed that s1/m1 type of vacA is more active than the s1/m2, and s2/m2 type does not produce detectable activity (4-5). Different studies have shown that s1/m1 genotype and the presence of *cagA* gene is associated with high degrees of colonization, inflammation, and neutrophilic activity when compared to s2/m2 genotype and the absence of *cagA* (5). It is therefore tempting to speculate that combination of bacterial and host genetic polymorphisms are involved in clinical manifestations of *H. pylori* infections. Interestingly when the patients were categorized according to the *cagA* status of infecting bacteria, only in patients who were infected with $cagA^-$ strains, a significant increase in myeloperoxidase -463 GG genotype frequency was found in peptic ulcer patients compared to patients with gastritis (table 2; p=0.0001). An intriguing question that arises from this finding is why the effect of different myeloperoxidase genotypes on clinical manifestation of infection was dependent on the absence of *cag*A gene in infected patients. Considering the ability of $cagA^+$ strains in neutrophil recruitment and activation through the induction of IL-8 (24), it could be hypothesized that in the case of infection with $cagA^+$ strains, regardless of myeloperoxidase -463 genotypes, the level of myeloperoxidase is high enough to enhance the chance of peptic ulcer development. Our findings also reveal a significant association between combined *vac*A and myeloperoxidase genotypes and clinical outcome of the disease. Indeed, association of myeloperoxidase -463GG genotype with peptic ulcer could be only detected in patients infected with *vac*A s1/m1 genotype (table2; p=0.008). This observation also indicates that in prediction of the clinical outcome of infection with *H. pylori*, simultaneous consideration of both bacterial and host genetic factors could provide more accurate information.

In conclusion**,** our findings suggest that the myeloperoxidase G-463A polymorphism could affect the clinical outcome of *H. pylori* infection. Furthermore, considering the combined bacterial and host genetic factors, a better picture of disease presentation after *H. pylori* infection could be obtained.

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