PO Box 2345, Beijing 100023, China
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World J Gastroenterol 2007 February 28; 13(8): 1243-1246
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Effects of the myeloperoxidase 463 gene polymorphisms on development of atrophy in *H pylori* infected or noninfected gastroduodenal disease

Ömer Yilmaz, Hakan Dursun, Nesrin Gürsan, İbrahim Pirim, Arif Yılmaz, Nihat Okcu

Ömer Yilmaz, Hakan Dursun, Arif Yılmaz, Nihat Okcu, Department of Gastroenterology, Faculty of Medicine, University of Atatürk, Erzurum 25070, Turkey
Nesrin Gürsan, Department of Pathology, Faculty of Medicine, University of Atatürk, Erzurum 25070, Turkey
İbrahim Pirim, Department of Medical Biology and Genetics, Faculty of Medicine, University of Atatürk, Erzurum 25070, Turkey
Correspondence to: Ömer Yilmaz, MD, Atatürk University, Medical Faculty, Department of Gastroenterology, Erzurum 25070, Turkey. yilmazo@atauni.edu.tr
Telephone: +90-442-2361212 Fax: +90-442-2361301
Received: 2006-11-03 Accepted: 2006-12-18

Abstract

AIM: To investigate the relationship between myeloperoxidase polymorphisms as a host-related factor and atrophy caused by *H pylori*.

METHODS: Our study enrolled 77 patients. Biopsy materials obtained during gastrointestinal endoscopies were evaluated for the presence of *H pylori*. Polymerase chain reaction-restriction fragment length polymorphism assay was used to characterize myeloperoxidase genotypes.

RESULTS: Forty four patients (57.1%) were *Hp* (+) and 33 (42.9%) were *Hp* (-). Sixty six (85.7%) had GG genotype, 10 (12.9%) had GA genotype and 1 (1.29%) had AA genotype. The change in atrophy in relation to neutrophil infiltration was significant in *Hp* (+) patients (P = 0.0001). The change in atrophy in relation to neutrophil infiltration in patients with GG genotype was significant (P = 0.002). However, the change in atrophy in relation to neutrophil infiltration was not significiant in patients with *Hp* (+) GG genotype (r = 0.066, P = 0.63).

CONCLUSION: Myeloperoxidase genotype is critical for development of atrophy in relation to the severity of inflammation. However, it is interesting to note that, *H pylori* does not show any additive effect on development of atrophy.

Key words: Gastritis; Gastroduodenal ulcer; Gastric cancer; Myeloperoxidase; *H pylori*

Yilmaz Ö, Dursun H, Gürsan N, Pirim İ, Yılmaz A, Okcu N. Effects of the myeloperoxidase 463 gene polymorphisms on development of atrophy in *H pylori* infected or noninfected gastroduodenal disease. *World J Gastroenterol* 2007; 13(8): 1243-1246

http://www.wjgnet.com/1007-9327/13/1243.asp

INTRODUCTION

H pylori is an agent that produces chronic infection in more than half of the world population[1]. The most important characteristic of *H pylori* infection is that it causes chronic active inflammation in the gastric mucosa, which involves neutrophils and monocytes[2]. While 100% of the persons carrying this microbe develops gastritis, the lifetime risk of peptic ulcer is 15%-20% and that of gastric cancer is 1%-3%[1].

The role of host-related factors in the pathogenesis of diseases caused by *H pylori* has been greatly ignored up to date[3,4]. It was demonstrated that the ability of the host to regulate the production of cytokines is influenced by the presence of polymorphisms in the promoter region of the relevant genes[5]. The polymorphisms in the genes affecting the production of cytokines might be one of the factors that lead to the interpersonal differences in the severity of gastric inflammation[3,5].

Myeloperoxidase (MPO) is a lysosomal enzyme found in the azurophilic granules of polymorphonuclear leukocytes (PNL)[6-9]. There are two promoter regions affecting MPO; -463G/A and -129G/A[10]. Allele A of this polymorphism reduces mRNA expression and thus, tissue damage in local inflammation is decreased[8,9,11]. Allele G has 25 times more transcriptional efficacies compared to allele A. The G/G genotype confers higher risk for persistent *H pylori* infection[12,13].

H pylori activates the oxidative metabolism in neutrophils[14]. Reactive oxygen products such as free oxygen radicals released from neutrophils, (O^{2-}), H_2O_2 and hydroxyl ion (OH) reacts with MPO and hypochloric acid (HOCI) is formed. Monochloroamines (NH_2CI) are formed when HOCI reacts with the ammonium produced by the urease enzyme of *H pylori*. Monochloroamines are oxidizing agents that are able to induce DNA fragmentation[4,8,14-16]. Thus, it has been advocated that

myeloperoxidase is involved in gastric damage induced by *H pylori*[4,11,17]. We assessed the association between neutrophil infiltration and atrophy caused by *H pylori* and MPO gene polymorphisms.

MATERIALS AND METHODS

We enrolled 77 patients (46 males, 31 females) who had undergone endoscopic examinations due to epigastric pain, dyspepsia, nausea and vomiting or weight loss. Patients who received antibiotic therapy, proton-pump inhibitors or non-steroid anti-inflammatory drugs within 3 mo prior to endoscopies were excluded.

Based on the endoscopic findings, 24 patients had gastritis, 26 had ulcers (duodenal or gastric ulcers) and 27 had gastric cancers. Diagnosis of patients who showed malignant findings in their endoscopies was confirmed by pathologic examination. In the light of endoscopic findings, two biopsies were obtained each from the antrum, angulus and corpus mucosa of the tissue adjacent to ulcer region and regions distant to malignant lesions. Biopsy materials were transferred into 3 separate small bottles containing formaldehyde and sent to the laboratory for evaluation of *H pylori* and pathologic examinations. Also, peripheral blood samples (3 mL) were collected from enrolled patients simultaneously and transferred into EDTA hemogram tubes. The tubes were transferred to the genetic laboratory under appropriate conditions avoiding coagulation.

Histopathologic examinations

The materials brought to the pathology laboratory were embedded in paraffin after follow-up procedures. Subsequently, cross-sections of 3-4 microns thick were obtained from the paraffin blocks. These cross-sections were stained by hematoxylin-eosin, Giemsa and Warthin-Starey stains, respectively. Preparations were divided into two groups as negative or positive based on the presence of *H Pylori* during direct visualization under a light microscope. Biopsy materials were staged according to neutrophilic activity, chronic inflammatory cell infiltration, glandular atrophy, intestinal metaplasia and *H pylori* density using criteria from the modified Sydney classification system. Each feature was evaluated as none (0), mild (1), moderate (2) or apparent (3)[18]. After all patients were scored according to 4 parameters of Sydney classification, sum of the parameters was calculated for three biopsy regions.

Myeloperoxidase genotyping

Patient blood samples transferred into EDTA tubes were used for DNA isolation. DNA isolation was performed using the column method (Gentra DNA isolation kit). Myeloperoxidase polymorphism analysis was performed by PCR and restriction fragment length polymorphism (RFLP) methods. Primers to be used were designed to detect Codone 463 of the myeloperoxidase gene.

Preparation of DNAs for PCR

Twenty microlitres DNA from each patient was transferred into 0.2 mL Eppendorf tubes. After addition of two different primers (forward pimer 5'-CCGTATAGGCAGA GAATGGTGAG-3' and reverse primer 5'-GCAATGGT TCAAGCGATTCTTC-3'), 1.5 μL; dNTP mix, 1 μL; Taq DNA polymerase, 1 μL; PCR buffer, 10 μL and distilled water, 15 μL, these tubes were placed into a PCR machine. PCR conditions were primer annealing at 56℃ for 1 min, polymerization at 72℃ for 1 min, and denaturation at 94℃ for 1 min. Thirty cycles were carried out[19]. The PCR product (amplicon) was incubated with *Acl*-I enzyme at 37℃ for 1.5 h and separated on a 2% agarose gel. DNA fragments on the gel were visualized after staining with 0.5 μg/mL ethidium bromide (EtBr).

Table 1 Distribution of myeloperoxidase genotypes in *Hp* (+) and *Hp* (-) patients according to endoscopic findings

	Hp (+)	*Hp* (-)	Total
n (%)	44 (57.1)	33 (42.9)	77 (100.0)
Gastritis	13	11	24
Ulcer (gastric or duodenum)	18	8	26
Gastric cancer	13	14	27
Myeloperoxidase GG	37	29	66
Myeloperoxidase GA	7	3	10
Myeloperoxidase AA	-	1	1
Neutrophil infiltration	2.80 ± 1.94[1]	0.88 ± 1.24	1.97 ± 1.91

Hp (+): *H pylori* positive group; *Hp* (-): *H pylori* negative group; $P < 0.0001$, *vs* the *Hp* (-) group. [1]Mean ± SD of a score of 0-3 according to the updated Sydney system.

Statistical analysis

Statistical analyses were performed using SPSS 11.0 for Windows statistical software, Kendall's Tau test and Linear regression analysis. P values < 0.05 were considered significant.

RESULTS

The mean age of the patients was 54.9 ± 14.1 years (19-82). Pathologic examination revealed that 44 of them were *Hp* (+) and 33 were *Hp* (-). Based on MPO gene polymorphism, 66 patients (85.7%) had GG genotype, 10 (12.9%) had GA and 1 (1.29%) had AA genotype. The patient with genotype A was an *Hp* (-) ulcer patient (Table 1).

There was a difference between *H pylori* (+) and *H pylori* (-) patients with respect to PNL infiltration (P = 0.0001). The change in atrophy in relation to neutrophil infiltration was significant in *Hp* (+) patients (y = 0.700x + 0.051) (P = 0.0001) (Figure 1). No such correlation was found for *Hp* (-) patients (y = 0.1214x + 0.50) (y: atrophy, x: neutrophil infiltration) (Figure 2) .

We found a significant correlation between atrophy and neutrophil infiltration in patients with GG genotype (n = 66) (y = 0.252x + 0.239) (P = 0.002). There was an insignificant correlation between atrophy and neutrophil infiltration in patients with GA genotype (n = 10) (y = 0.15x + 1.387) (P = 0.56).

Among the *Hp* (+) patients, the correlation between atrophy and neutrophil infiltration was insignificant for patients with both GG (n = 37) and GA genotypes (n = 7) (r = 0.066, P = 0.63; r = -0.474, P = 0.18, respectively).

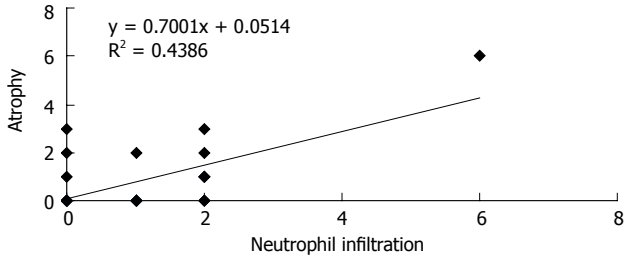

Figure 1 Changes in the atrophy in relation to neutrophil infiltration in *Hp* (+) patients.

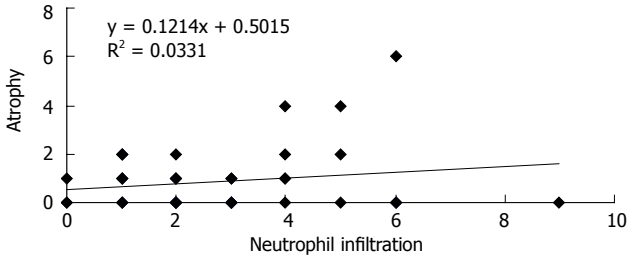

Figure 2 Changes in the atrophy in relation to neutrophil infiltration in *Hp* (-) patients.

Among the patients with *Hp* (-), we found an insignificant correlation between atrophy and neutrophil infiltration in patients with both GG (n = 29) and GA genotypes (n = 3) (r = 0.316, P = 0.06; r = 0.816, P = 0.22, respectively). Since our sample size was small, a statistical analysis for MPO AA (n = 1) genotype could not be performed.

DISCUSSION

About 60% of the world population is infected with *H pylori*[1]. Hyperproliferation induced by *H pylori* gastritis has been assumed as a starting point for events that trigger gastric cancer. Moreover, this hyperproliferation has been suggested to initiate changes in DNA[4,20,21]. The World Health Organization (WHO) has declared *H pylori* as the leading carcinogenic factor. In a cohort study conducted by WHO in 3 different regions, anti-*Hp* antibody levels were measured in blood samples obtained within the last 24 years from patients diagnosed with gastric cancer. It was stated that *H pylori* seropositivity increased the relative risk for developing gastric cancer[1]. Yamagato *et al*[22] reported that 3% of 1721 patients with *H pylori* developed gastric cancer during 9 years of follow up. Also, Uemura *et al*[23] reported that 2.9% of 1246 *Hp* (+) patients developed gastric cancer during 7.6 years of follow up. While there was a significant difference in *Hp* (+) patients with respect to atrophy and neutrophil infiltration, no such correlation was found for *Hp* (-) patients. Thus, inflammation induced by *H pylori* could be one of the predisposing factors in carcinogenesis.

Recently studies have been published on cytokine production in several conditions caused by *H pylori* infection as a host response and genetic polymorphisms[4]. Zambon *et al*[3] found that bacterial and virulence factors were associated with mucosal inflammation and severity of the illness. They found that *H pylori* virulence genes and a host genotype of IL-1 RN were directly correlated with peptic ulcer and intestinal metaplasia and they suggested that the interaction between cytokine genotypes and bacterial virulence factors are fundamental to development of *H pylori*-related lesions. Nardone *et al*[24] reported that, first atrophy localized in the antrum regresses or disappears subsequent to *H pylori* eradication. The changes seen in these lesions as a result of eradication show that, neutrophil infiltration plays a major role in these events above all.

Polymorphisms, defined as known changes in the genomic sequence, occur frequently throughout the human genome. It is known that in some cases, they modify the expression or function of gene products. Any polymorphism could affect susceptibilities to and outcome of an illness through the interaction of environment and genetics[25].

The question of how the variations in the genes associated with inflammation affect the inflammatory response induced by *H pylori* and accompanying gastric pathologies is an interesting one[26]. It has been stated that MPO itself might modulate susceptibilities to clinical outcomes of several illnesses where neutrophils are involved[10]. In a number of studies, the association between MPO polymorphisms and several conditions including chronic granulomatous illness, Alzheimer' s disease, malignancies such as the lung or pharyngeal cancer, some types of leukemia, atherosclerosis, periodontal diseases, multiple sclerosis and cystic fibrosis were reported[4,8,11-13]. Hamajima *et al*[12] investigated a myeloperoxidase genotype with low expression, which was negatively correlated with *H pylori* infection in 241 patients having complaints of dyspepsia, without a history of cancer. They found 79.7% of GG genotype, 19.5% of GA genotype and 0.8% of AA genotype. In another study assessing the association between MPO polymorphisms, atrophy and neutrophil infiltration, Roe *et al*[4] found 81.9% of GG genotype, 18.1% of GA genotype and 0.0% of AA genotype. It was indicated that while there was a positive correlation between the degree of atrophy and neutrophil infiltration in individuals with GG polymorphism, no such association was found in individuals with GA polymorphism. Also, in our study we found that while the correlation between atrophy and neutrophil infiltration was significant in patients with GG genotype, it was insignificant in patients with GA genotype.

Our results show that generally myeloperoxidase GG genotype is a critical host-related factor for development of atrophy that occurs in the setting of inflammation, and is considered as a cancer precursor. However, it is interesting to note that on the contrary to what is expected, GG genotype and *H pylori* positivity does not potentiate each other for development of atrophy. More studies are needed to elucidate this point in larger patient groups.

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S- Editor Liu Y **L- Editor** Zhu LH **E- Editor** Ma WH