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Original Article

TLR2 Arg677Trp but not TLR2 -196 to -174 ins/del and Arg753Gln polymorphism alter the risk of peptic ulcer in north of Iran

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Abstract

Background: Most polymorphisms that occur in TLR-2 are associated with gastrointestinal disorders such as peptic ulcer disease (PUD). Hence, in current study, association between TLR2-196 to -174 ins/del, Arg753Gln and Arg677Trp polymorphisms and risk of PUD development in north of Iran was evaluated.

Methods: This case-control study included 50 patients with PUD as cases and 50 people without peptic ulcer as control group. Blood and endoscopic biopsies were collected. *Helicobacter pylori* infection was screened by rapid urease test, specific IgG measurement and specific PCR for glmM gene. Then, TLR2-196 to -174 ins/del polymorphism was assessed by using allele-specific PCR. The Arg753Gln and Arg677Trp polymorphism in TLR2 gene were analyzed by the PCR-restriction fragment length polymorphism (RFLP).

Results: There was no significant difference in the allele and genotype frequencies of polymorphisms in the TLR2-196 to -174 ins/ins and Arg753Gln genes between controls and patients, respectively. However, an association with increased risk for PUD was observed for polymorphism TLR-2 Arg677Trp (odds ratio [OR] = 7.9; 95% confidence interval [CI] = 0.94−67.5). Further analysis showed that *H. pylori* infection was associated with a significant difference in genotype and allele frequencies of TLR2-196 to -174 ins/ins and Arg753Gln polymorphism, respectively. Furthermore, there was no association between variant haplotypes and PUD development in *H. pylori* infected subjects. However, no association was detected between gender and genotypic frequencies of all polymorphisms in TLR2.

Conclusion: Our findings showed that TLR2 Arg677Trp polymorphism and *H. pylori* infection may play crucial roles in peptic ulcer development respectively in north of Iran.

Keywords: Genetic polymorphism; Peptic ulcer; TLR2

1. Introduction

Peptic ulcer disease or PUD is one of the most common disease and some of its complications are main causes of morbidity and mortality in the world.[1] Although, around 4% of the world's population are suffered from PUD,[2] but its prevalence differs between countries; the Asian countries, has much higher incidence rates than the European countries.[3]

Peptic ulcer was defined as a circumscribed mucosal break of at least 0.5 cm in diameter and a perceptible depth.[4−6] It is characterized by an imbalance between the factors that damages the mucosa and those for its protection, resulting in a lesion of the lining of the upper digestive tract.[7]

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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The *Helicobacter pylori* (*H. pylori*) is major etiological factor for PUD that leads to inflammation of the gastric mucosa in 80% of peptic ulcer cases.[8] However, 50% of the world's population are infected with *H. pylori*, but a small percentage of those develop PUD and other gastric diseases.[9] It seems, other factors such as host genetic factors may play an important role in development of peptic ulcer.

Host genetic factors such as polymorphisms of Toll-like receptors (TLRs) and immune response gens can affect in magnitude the host immune response against infection and lead to the variation in the level of cytokine response that may influence in the susceptibility to inflammatory disease.[10–12] TLRs are pattern recognition receptors that play some critical roles in immune response against harmful pathogens.[13] The immune response against *H. pylori* initiate by expression of TLRs in the surface of infected gastric epithelial cells. These receptors can induce cellular signaling pathways including activation of nuclear factor (NF)-κ B and inflammatory cytokine production.[14]

Among the TLRs, most studies have shown that the TLR2 acts an initial barrier against *H. pylori* and is a much better receptor to recognize bacterial LPS.[15] Expression of TLR2 leading to activation of transcription factors, mainly NF-kB that induce the production of pro-inflammatory cytokines such as IL-1β, IL-2, IL-6, IL-8, IL-12.[9] TLR2 activation also leads to expression of TLR4 and proliferation of gastric epithelial cells to metaplasia, to dysplasia, and adenocarcinoma.[16,17]

Single nucleotide polymorphisms (SNPs) occur in TLR2 gene have been associated with an increased susceptibility to various infectious and inflammatory diseases such as asthma, tuberculosis, lepromatous leprosy and septic shock in different population.[18,19] These polymorphisms can influence on the pathogenesis of inflammatory diseases. It can be cause of variation in expression of pro-inflammatory cytokines genes and increase of inflammatory response.[20] Therefore, increases inflammatory response can lead to atrophy and eventually ulcers in the stomach and also may increase the risk of stomach cancer.[21]

Recently, TLR2-196 to -174 ins/del polymorphism was found to be associated with susceptibility to intestinal metaplasia[14] and gastric cancer.[8] Studies demonstrated the TLR2-196 to -174 ins/del polymorphism may influence TLR2 promoter activity. It has been reported that the TLR2-196 to -174 del/del genotype showed decreased transactivation of responsive promoters. It is considered that this polymorphism associated with gastro-duodenal disease.[22] Likewise, reported that TLR2 gene (Arg677Trp and Arg753Gln) polymorphisms have been associated with an increased susceptibility to various diseases such as, tuberculosis, lepromatous leprosy and septic shock.[18,23,24]

Therefore, polymorphisms in TLR2 gene may plays a key role in susceptibility to PUD. Hence, in the current study, we investigated role of the genetic variants of TLR2-196 to -174 ins/del, Arg753Gln and Arg677Trp in susceptibility to peptic ulcer in north of Iran.

2. Methods

2.1. Patients and samples

In this case-control study, patients with dyspepsia who underwent endoscopy at Ayatollah Rohani Hospital (Babol University of Medical Sciences, Babol, Iran) between January 2016 and September 2016 were enrolled. The demographic data, information about symptoms of abdominal pain, and data regarding history of chronic stomach disorders were obtained via a standardized questionnaire. Patients receiving antimicrobial therapy, proton-pump inhibitors, H_2-receptor blockers, and non-steroidal anti-inflammatory drugs were excluded. All biopsy samples were taken from the gastric antrum. Three biopsy samples were taken from each patient during endoscopy. One of the biopsy samples was applied for the rapid urease test and the second one processed for histopathological examination. The third biopsy sample was preserved in transport medium for *H. pylori* detection via PCR. Based on the endoscopic observation by an expert gastroenterologist and histopathological assessments, subjects were divided into two groups including peptic ulcer (PUD) as case and non-peptic ulcer (NPUD) including normal or gastritis as control group. The Research Ethics Committee of Babol University of Medical Science approved this work (MUBABOL.REC.1394.106), and written informed consent was obtained from all participating individuals.

2.2. Detection of H. pylori infection

The presence of *H. pylori* infection was determined on the basis of histopathological examination including Giemsa staining, rapid urease test (RUT), and anti-*H. pylori* IgG antibody. Briefly, anti-*H. pylori* IgG antibody was measured in serum samples of the participants with a direct enzyme-linked immunosorbent assay (ELISA) kit (Pishtaz Teb, Tehran, Iran). According to the manufacture instructions, a finding of 10.0 IU/ml or higher was regarded as seropositive. In addition, to proving the presence of *H. pylori* in the biopsy samples, the *Helicobacter* species-specific PCR were performed using specific primers for the ureC (glmM) gene as previously described.[25] Patients were considered infected with *H. pylori* if the results were positive by at least two methods.

2.3. Genotyping for TLR2-196 to -174 ins/del polymorphism

Peripheral venous blood was collected from all participants in EDTA containing tubes, and genomic DNA was extracted using the salting-out procedure.[26] DNA was stored at −20 °C until use for genotyping. Allele-specific polymerase chain reaction (ASPCR) method was applied to investigate -196 to -174 ins/del polymorphism. The nucleotide sequences of the sense and antisense primer was described in Table 1. In brief, the PCRs were performed separately in 20 μl reaction volume containing 50 ng of genomic DNA, 12.5 pmol of each primer,

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