POLYMORPHISMS OF THE IL-6, IL-8 AND IL-10 GENES AND THE RISK OF GASTRIC PATHOLOGY IN PATIENTS INFECTED WITH HELICOBACTER PYLORI

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Background/Purpose: Helicobacter pylori-induced gastric mucosal inflammation is mediated by proinflammatory and anti-inflammatory cytokines. Polymorphisms in genes that code cytokines influence cytokine secretion levels and appear to contribute to the risk of gastric diseases. In this sense, we performed this study to identify the polymorphisms in the IL-6, IL-8, and IL-10 genes and their associations with H. pylori infection and gastric pathologies.

Methods: Gastric biopsy samples of 151 patients infected with H. pylori and 76 uninfected individuals were used. Helicobacter pylori infection was diagnosed by histological examination and the detection of the ureA and glmM genes. The polymorphisms in the IL-6 (at position –174), IL-8 (at position –251), and IL-10 (at position –819) were detected by polymerase chain reaction—restriction fragment length polymorphism.

Results: Among the genetic polymorphisms studied, we observed that only the presence of the A allele at position –251 of the IL-8 gene was significantly associated with H. pylori infection. In addition, patient carriers of the A/A genotype at position –251 of the IL-8 gene and carriers of the T allele at position –819 of the IL-10 gene had an increased risk of peptic ulcer disease in the presence of H. pylori infection. We did not find a correlation between polymorphisms in the IL-6, IL-8, and IL-10 genes and a higher risk of gastric carcinoma.

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Introduction

*Helicobacter pylori* is a bacterium that causes various diseases such as chronic gastritis, peptic ulcer disease, gastric carcinoma, and mucosa-associated lymphoid tissue lymphoma. The key pathophysiological event in *H. pylori* infection is the induction of an inflammatory response in the gastric mucosa, which is mediated and regulated by inflammatory cytokines produced by epithelial cells. Polymorphisms in genes encoding cytokines such as interleukin (IL)-6, IL-8, and IL-10 influence the cytokines’ secretion levels and appear to contribute to the risk of developing gastroduodenal diseases.

Interleukin-6 is a proinflammatory cytokine that functions as an inflammatory mediator and an endocrine regulator. In addition, it has an important role in host defense mechanisms as a messenger between innate and adaptive systems. The IL-6 gene is located on chromosome 7. A polymorphism in the 5’ flanking region at position −174 (G→C) has been described. This polymorphism may result in interindividual variations in the transcription and expression of the IL-6 gene, and therefore influence an individual’s susceptibility to a diverse range of diseases, which include *H. pylori* infection, and underlying pathogenesis. The gastric mucosal levels of IL-6 are elevated in *H. pylori*-associated gastritis and diminished after the eradication of the infection. In addition, gastric levels of IL-6 are reportedly increased in *H. pylori*-positive individuals with gastric carcinoma.

Interleukin-8 is another proinflammatory cytokine that has an important role in the pathogenesis of *H. pylori*-induced diseases. The high expression of IL-8 has been demonstrated in gastric mucosa infected with *H. pylori*. IL-8 causes chemotaxis and the activation of inflammatory cells in gastric mucosa infected with *H. pylori*. The IL-8 gene, which is located on chromosome 4, exhibits a single nucleotide polymorphism (SNP T→A base transition) at −251 nt relative to the transcription start site. The A allele tends to be associated with increased IL-8 production and consequently with an amplified inflammatory response.

Unlike the two aforementioned cytokines, IL-10 is an anti-inflammatory cytokine that downregulates cell-mediated immune responses and cytotoxic inflammatory responses. *Helicobacter pylori* can lead to IL-10 upregulation as a way to suppress an efficient immune response, which then favors infection and parasite survival. The gene encoding human IL-10 is located on chromosome 1. Two SNPs that are associated with low IL-10 production have been reported in the promoter region of this gene: a C→T base transition at position −819 and a C→A base transition at position −592. The low IL-10 production is associated with increased gastric inflammation intensity and with an enhanced risk of gastric carcinoma in patients infected with *H. pylori*.

In this context, the identification of host genetic polymorphisms associated with the protection from or the induction of gastric disease in individuals of different ethnicities can allow a better prognosis of *H. pylori* infection. In the present study, we aimed to determine the frequency of polymorphisms in the IL-6, IL-8, and IL-10 genes in patients from the south region of Brazil, to analyze the association of these polymorphisms with *H. pylori* infection, and to investigate the relation between these polymorphisms and normal gastric mucosa, gastritis, peptic ulcer disease, and gastric carcinoma.

Methods

Patients and gastric biopsy samples

The present study included 227 patients (125 women and 102 men with an average age of 53.4 years): 151 *H. pylori*-positive patients (23 patients with a normal gastric mucosa, 86 patients with gastritis, 37 patients with peptic ulcer, and 5 patients with gastric cancer) and 76 *H. pylori*-negative patients (15 patients with a normal gastric mucosa, 45 patients with gastritis, 12 patients with peptic ulcer, and 4 patients with gastric cancer). The average age of the *H. pylori*-positive patients was 52.4 years and the average age of the *H. pylori*-negative patients was 54.4 years. Eight biopsy samples were obtained from each patient. Of these, four samples were destined for histology (two samples from the gastric antrum and two samples from the gastric body), whereas the remaining four samples were intended for polymerase chain reaction (PCR) (two samples from the gastric antrum and two samples from the gastric body). *Helicobacter pylori* infection was diagnosed by histology and by the detection of the *ureA* and *glmM* genes by PCR, as described later. Patients were considered as infected with the bacterium when positive results were obtained in at least two of the three tested methods and considered as uninfected when the results of all diagnostic tests were negative. The diagnosis of gastroduodenal diseases was based on endoscopic and histological examinations and established in accordance with the Sydney System Classification. This work was approved by the Research Ethics Committee of the Health Area [Universidade Federal do Rio Grande (FURG); process number 23116.001044/2011-16]. Written, informed consent was obtained from all patients.
Histology, DNA extraction, and PCR

Gastric biopsy samples that were destined for histology were fixed in 10% formalin after collection, and were stained with hematoxylin-eosin (H&E) and Giemsa. Microscopic examination defined the degree of involvement of the gastric mucosa and the presence of H. pylori. After collection, the samples that were intended for PCR were kept in brain heart infusion broth with 20% glycerol. They were then stored at –70°C for further extraction. The DNA was extracted using DNAzol Reagent (Invitrogen Life Technologies, Carlsbad, USA) and proteinase K (Ludwig Biotech, Porto Alegre, Brazil) and 10 μg/mL of proteinase K, as described previously by Fonseca et al.20 The integrity of the extracted DNA was assessed by the amplification of a 110-base pair (bp) fragment, which is specific to human β-globin, using initiator oligonucleotides and a methodology already reported in another study.21 Helicobacter pylori infection was diagnosed by PCR using two sets of primers: UREA1–UREA2, which amplifies a 394-bp fragment that corresponds to the ureA gene,22 and GLM/MF–GLM/MR, which amplifies a 140-bp fragment that corresponds to the glmM gene.23

Genotyping of cytokine polymorphisms

Polymorphisms in the IL-6, IL-8, and IL-10 genes were detected by PCR-restriction fragment length polymorphism (RFLP). To investigate the polymorphism at position –174 of the IL-6 gene, we used the primers 5′-TTGTCAAGACTGCGAACTTTG-3′ (forward) and 5′-TCAGATCTCTCAA GTCCATA-3′ (reverse).24 The PCR was performed under the conditions described by Gatti et al.24 The PCR products were digested with the restriction enzyme MfeI, and then analyzed by electrophoresis on 3.5% agarose gel stained with ethidium bromide. The genotypes were designated as follows: T/T, a single band consisting of 108 bp; T/A, three bands consisting of 108 bp, 76 bp, and 32 bp; and A/A, two bands consisting of 76 bp and 32 bp.25

To determine the polymorphism at position –819 of the IL-10 gene, primers 5′-ATCCAGACAAACTCTTAA-3′ (forward) and 5′-TAAATATCCTCAAAGTTCC-3′ (reverse) were used.26 The PCR was performed under the conditions described by Cheng et al.26 The PCR products were digested with the restriction enzyme MaeIII, and then analyzed by electrophoresis on 2.5% agarose gel stained with ethidium bromide. The genotypes were designated as follows: C/C, three bands consisting of 292 bp, 217 bp, and 79 bp; C/T, four bands consisting of 509 bp, 292 bp, 217 bp, and 79 bp; and T/T, two bands consisting of 509 bp and 79 bp.26 To ratify the results obtained by PCR-RFLP for the IL-6, IL-8, and IL-10 genes, 10% of samples evaluated for each polymorphism were randomly selected and confirmed by DNA sequencing.

Statistical analysis

The sample size was calculated using Epi-info 6.04 software (Centers for Disease Control and Prevention, Atlanta, GA). A 95% confidence level was used. The estimated prevalence of the IL-6 polymorphism was 8% with an error of 4%. We choose this polymorphism for the calculation of the "n“ because is the less frequent among the studied. We found 193 patients added of 15% of loss. The data was typed on a bank in the Microsoft Excel 2010 program (Microsoft Corporation, Redmond, Washington, USA). The consistency analysis, which was based on the creation and categorization of variables and verification of frequencies, was performed using SPSS version 18.0 (Statistical Program for the Social Sciences Inc., Chicago, USA). The Chi-square test or Fisher’s exact test were used to compare between proportions. Values of p < 0.05 were considered statistically significant. A prevalence ratio (PR) with a 95% confidence interval (CI) was calculated to evaluate the relationship between cytokine gene polymorphisms with gastroduodenal diseases and with H. pylori infection.

Results

Table 1 shows the allelic and genotypic frequency refers to polymorphisms in the IL-6, IL-8, and IL-10 genes in H. pylori-positive and H. pylori-negative patients. Only the presence of the A allele at position –251 of the IL-8 gene was significantly associated with H. pylori infection (p = 0.039).

Table 2 displays the distribution of the allele and geno-type referents to polymorphisms in the IL-6, IL-8, and IL-10 genes in H. pylori-positive and H. pylori-negative patients with a normal gastric mucosa, gastritis, peptic ulcer disease, and gastric carcinoma. Helicobacter pylori-positive patients and carriers of the A/A genotype at position –251 of the IL-8 gene had an increased risk of peptic ulcer disease (PR = 2.08 and 95%CI = 1.03–4.18). In addition, patient carriers of the T allele at position –819 of the IL-10 gene in the presence of H. pylori infection had a higher risk of peptic ulcer disease (PR = 1.24 and 95%CI = 1.01–1.53).

Discussion

Allelic variants in cytokine genes influence gene expression and susceptibility to infectious diseases.27 Naito et al11 showed that the IL-8 levels in A allele carriers of the IL-8 T-251A polymorphism were slightly higher than in subjects with the T/T genotype. In this study, unlike some previous works, the presence of the A allele at position –251 of the IL-8 gene was significantly associated with H. pylori infection (p = 0.039), which suggests that this allele is associated with susceptibility to H. pylori infection and its persistence.13,28 Xue et al13 showed in a meta-analysis that the IL-8 -251AA genotype is not associated with the presence of the A allele at position -251 of the IL-8 gene was significantly associated with IL-8, IL-10 genotypes, and the presence of H. pylori infection status. However, Xue found that this genotype is associated with the overall risk of developing gastric cancer. In 2013, Zhao et al28 investigated the correlation...
between \textit{H. pylori} infection and the host genetic background of healthy populations in Indonesia. Zhao did not observe a statistical significance between \textit{IL-8}, \textit{IL-4}, \textit{IL-13}, \textit{CD14}, \textit{TNF-\textalpha}, and tyrosine-protein phosphatase nonreceptor type 11 (PTPN11) gene polymorphisms and \textit{H. pylori} infection. \textit{H. pylori}-induced gastric mucosal inflammation is mediated by an array of pro- and anti-inflammatory cytokines.\textsuperscript{3} In general, studies show that this inflammation is mediated by an array of pro- and anti-inflammatory cytokines, which result in a higher risk of peptic ulcer or gastric carcinoma.\textsuperscript{3} Ulcer occurs because of a disequilibrium between defensive mucosa-protective factors and aggressive injurious factors; carcinogenesis occurs because of the accumulation of genetic alterations and the dysfunction of cellular mechanisms that normally maintain human genome integrity.\textsuperscript{29,30}

In accordance with a previous report, \textit{H. pylori}-positive patients and carriers of the A/A genotype at position \textasciitilde{251} of the \textit{IL-8} gene had an increased risk of peptic ulcer disease.\textsuperscript{31} Allele A is associated with higher \textit{IL-8} production, and consequently with increased mucosal injury, by activating and recruiting neutrophils in response to infection by \textit{H. pylori}.\textsuperscript{32,33} However, unlike some papers that have reported that polymorphisms in the \textit{IL-10} gene were not associated with peptic ulcer, the current study concluded the opposite.\textsuperscript{34,35}

After analyzing gastric biopsy samples from 227 patients, we found that patient carriers of the T allele at position \textasciitilde{819} of the \textit{IL-10} gene had a higher risk of peptic ulcer disease in the presence of \textit{H. pylori} infection. This allele is associated with the decreased expression level of \textit{IL-10}, which stimulates the proinflammatory response.\textsuperscript{1} In 2010, Cheng et al\textsuperscript{34} demonstrated among Taiwanese patients that the \textit{IL-10} \textasciitilde{819}T/T genotype among patients infected with \textit{H. pylori}-infected Koreans. Zambon et al\textsuperscript{34} showed that the \textit{IL-10} \textasciitilde{819}T/T genotype among Italian patients was associated with noncardia gastric cancer, but not with peptic ulcer. Despite the low sample size, we did not find a correlation between polymorphisms in the \textit{IL-6}, \textit{IL-8} and \textit{IL-10} genes and a higher risk of gastric carcinoma as Savage et al,\textsuperscript{36} Alpizar-Alpizar et al,\textsuperscript{37} Savage et al.\textsuperscript{38} However, other authors have reported a correlation.\textsuperscript{32,34,39} Ye et al\textsuperscript{32} observed that the \textit{IL-8} \textasciitilde{251}T/T allele seemed to increase the risk of gastric adenocarcinoma through an enhanced inflammatory process in \textit{H. pylori}-infected Koreans. Zambon et al\textsuperscript{34} showed that the \textit{IL-10} \textasciitilde{819}T/T genotype among Italian patients was associated with noncardia gastric cancer. These contradictory results may be related to genetic and ethnic differences in populations and with differences in the study designs, in the sample size used, in the patients’ age at diagnosis, and in the dietary habits. The magnitude and direction of the inflammatory response is directed by host genetic factors interacting with environmental exposures.\textsuperscript{40}

In 2009, Kang et al\textsuperscript{41} evaluated the same genetic polymorphisms that we studied and their associations with...
<table>
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The p values were determined by Fisher's exact test.

The p values were determined by the Chi-square test.

95% CI, 95% confidence interval; H. pylori, Helicobacter pylori; IL-6/-8/-10, interleukin-6/-8/10; PR, prevalence ratio.
gastroduodenal disease in a Korean population. They found a significant positive association between the IL-8 -251A/A genotype and benign gastric ulcer. Furthermore, a synergistic effect was observed between IL-10 -592A/A and IL-8 -251A/A with respect to the development of gastric carcinoma and benign gastric ulcer.

From this study, we suggest that polymorphisms in the IL-8 and IL-10 genes are biologically important in the pathogenesis of peptic ulcer disease in patients infected with *H. pylori*. A limitation of this study was that the limited number of patients who were evaluated were diagnosed with peptic ulcer disease and principally with gastroduodenal disease in a Korean population. They found a significant positive association between the IL-8 -251A/A genotype and the risk for *H. pylori*-associated gastric carcinoma and benign gastric ulcer. A limitation of this study was that the number of patients who were evaluated were diagnosed with peptic ulcer disease and principally with gastroduodenal disease in a Korean population. They found a significant positive association between the IL-8 -251A/A genotype and the risk for *H. pylori*-associated gastric carcinoma and benign gastric ulcer.

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