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Polymorphisms and haplotypes of the interleukin 2 gene are associated with an increased risk of gastric cancer. The possible involvement of *Helicobacter pylori*

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ABSTRACT

Interleukin 2 (IL-2) is a pro-inflammatory cytokine that is mainly synthesized by immunoregulatory T helper cells and which plays an important role in antitumor immunity. *Helicobacter pylori (H. pylori)* is a gram-negative bacterium that colonizes the gastric mucosa and induces the production of IL-2. This process increases the magnitude of inflammation and may influence the development of gastric pathologies. In light of the possible involvement of IL-2 and the presence of *H. pylori* in gastric diseases, this study investigated possible associations between the IL-2 polymorphisms +114 T > G (rs2069763) and −330 T > G (rs2069762) and the development of gastric cancer; these associations were then correlated with the presence of *H. pylori*. Gastric biopsies were obtained from 294 dyspeptic patients (173♀/123♂). Of these samples, 181 were chronic gastritis samples (102♀/79), 62 were samples of intact gastric mucosa (47♀/15♂), and 51 were samples of gastric cancer (22♀/29♂). PCR-RFLP was used to characterize the +114 T > G and −330 T > G polymorphisms. Considering the genetic characteristics of the study population and based on the codominant model, a high risk of gastric cancer among patients with normal gastric tissue and patients with gastric cancer was found in subjects with the IL-2-330 GG genotype (OR = 6.43, 95% CI: 1.47–28.10, $p = 0.044$). The data was adjusted for the presence of *H. pylori*. Among patients with gastritis and patients with gastric cancer, a high risk was found among subjects with the IL-2-330 GG genotype (OR = 4.47, 95% CI: 1.84–10.84, $p = 0.0022$). When the IL-2 +114 polymorphism was analyzed, similar results were found. Among the patients with normal gastric tissue and the patients with gastric cancer, subjects carrying the +114 TT genotype were found to be at a high risk of gastric cancer (OR = 5.97, 95% CI: 1.60–22.27, $p = 0.013$). This data was also adjusted for the presence of *H. pylori*. Among patients with gastritis and patients with gastric cancer, a high risk was found in subjects carrying the +114 TT genotype (OR = 6.36, 95% CI: 2.66–15.21, $p < 0.0001$). The haplotype was also analyzed. The −330G/+114T haplotype was found to be significantly associated with gastric cancer. Therefore, our results show that, among patients with *H. pylori* infection, the −330 GG and +114 TT genotypes are significantly associated with a high risk of developing gastric cancer, as is the −330G/+114T haplotype.

1. Introduction

Helicobacter pylori (*H. pylori*) is a spiral-shaped, flagellated, microaerophilic, gram-negative *bacillus* bacterium that colonizes the gastric mucosa and which is the most common cause of gastric diseases such as chronic gastritis, peptic ulcers, and gastric cancer [1,2]. Gastric cancer is the fourth most common cancer in the world and the second leading cause of cancer deaths [3]. Considering that *H. pylori* infects almost 50% of population, cause an intense inflammatory response and evades eradication, a significant percentage (25%) of the infected individuals go on to developing gastric cancer. According with Atherton [4] and Kalisperati, et al. [5] three important factors are associated with the development of symptomatic *H. pylori* disease: (1) *H. pylori's* virulence markers such as the *cagA*, *vac*A and *dup*A genes (2) host susceptibility and response (including genetics aspects such polymorphisms in interleukins, epigenetic factors and gene expression) and (3) environmental cofactors, including smoking and diet [6,7].

The main pathophysiological event of *H. pylori* infection in the

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gastric mucosa is the induction of an inflammatory response resulting from the contact between the bacterium and gastric cells. This contact activates the neutrophils and mononuclear cells that synthesize several pro-inflammatory cytokines, including IL-2 [8,9]. Increased production of inflammatory cytokines in response to *H. pylori* infection results in severe gastric inflammation that may influence the development of gastric disease [10].

Interleukin 2 (IL-2) is a pro- and anti-inflammatory cytokine that acts as immunoregulator. It is involved in cell-mediated immune response [11,12]. This cytokine has been identified as an autocrine secretory product from activated T cells that is coded by a single gene located on chromosome 4q21. It contributes to the proliferation of regulatory T cells and regulates expansion and apoptosis among activated T cells [13,14]. The IL-2 gene is characterized by single nucleotide polymorphisms (SNPs) such as −330 T > G (rs2069762) in the promoter region and +114 T > G (rs2069763), which is located on the first exon, suggesting associations with various types of cancer [14,15]. Studies on IL-2 gene polymorphisms have reported that these polymorphisms are important in regulating the rate of inducible expression and secretion of IL-2 [15].

The IL-2 polymorphism −330 T > G has been associated with increased susceptibility to a range of inflammatory diseases and cancers, including gastric atrophy from *H. pylori* infection and gastric cancer [16]. Studies have reported the G allele to be associated with reduced IL-2 production in individuals; it may downregulate antitumor response and may also play a role in the etiology of cancer [17–19].

Melo Barbosa, et al. [20] described that polymorphisms in the promoter region of the gene, such as IL-2-330, have been associated with an increase in the hypochlorhydria of gastric mucosa and the synthesis of interleukins; hypochlorhydria may be considered a risk factor for cancer [21]. In another study, Zhao and Wang [14] reported the IL-2-330 T > G polymorphism to be associated with an increased the risk of gastric cancer in Asian patients. Meanwhile, Wang, et al. [12] reported that the −330 T > G polymorphism is not associated with a risk of cancer. More recently, Zhang, et al. [15] suggested that no association exists between the +114 T > G polymorphism and the risk of cancer, and found only the −330 T > G polymorphism to be associated.

As mentioned previously, immune response may be considered a key event in the pathogenic process that leads to gastric disease. Therefore, this study investigated possible associations between the IL-2 polymorphisms −330 T > G (rs2069762) and +114 T > G (rs2069763) and the development of gastric disease; these associations were then correlated with the presence of *H. pylori*.

2. Materials and methods

2.1. Patients and protocol

This study evaluated a total of 294 samples of dyspeptic patients (173♀/123♂, mean age 53.99 ± 1455 years). A total of 181 samples were from patients with chronic gastritis (102♀/79♂, mean age 53.30 ± 14.47 years), 62 samples were from patients with intact gastric mucosa (47♀/15♂, mean age 53.71 ± 14.58), and 51 samples were from patients with gastric cancer (22♀/29♂, mean age 59.33 ± 14.07).

All groups had similar ethnic origins: 80% were of European descent, 2.5% were of Japanese descent, 1.5% were Amerindian, 5% were blacks and “pardo” (brown-skinned) and about 11% were multi-ethnic.

These gastric biopsies from patients with gastric symptoms were obtained in collaboration with the State Hospital of Bauru (HEB). Samples from gastric cancer patients were obtained in collaboration with the Federal University of São Paulo (UNIFESP). The patients who had undergone antimicrobial therapy treatment and/or had received treatment via proton pump inhibitors and/or had used NSAIDs in the three months prior to the endoscopy were excluded from the study.

This study was submitted to and approved and by the Ethics and Research Committee of Sagrado Coração University located in Bauru, São Paulo State, Brazil (Nos. 1.006.600 and 1.045.181).

2.2. DNA extraction and analysis of IL-2 polymorphisms

The extraction of DNA from the gastric biopsies of the antral region of stomach was performed according to the protocol established by the Qiagen QiaAmp Kit (Qiagen, Germany).

In order to determine the −330 (T > G) and +114 (T > G) polymorphisms the restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) technique was performed according to the conditions described by Sayad and Movafagh [22] and Song, et al. [16], respectively.

For the characterization of the −330T > G and +114T > G polymorphisms, fragments of 131 bp and 262 bp were amplified, respectively. After the PCR, the −330T > G and +114T > G amplicons were treated with *BfaI* and *MwoI* restriction enzymes, respectively, and the products were fractionated in agarose gel at 2.5%. They were then stained with ethidium bromide, displayed on an ultraviolet transilluminator, and photographed in order to analyze the distribution of alleles using the Alpha Imager 2200 imaging system (Alpha Innotech Coorporation).

In the case of the −330 T > G polymorphism, the amplification products were a 131-bp fragment (genotype TT), 131-bp, 110-bp and 21-bp fragments (genotype TG), and 110-bp and 21-bp fragments (genotype GG). In the case of +114 T > G, the products of amplification were: a 262-bp fragment (genotype TT), 262-bp, 113-bp, and 149-bp fragments (genotype TG) and 113-bp and 149-bp fragments (genotype GG).

2.3. Statistical analysis

Allelic and genotypic frequencies were estimated through the use of allele counts and the Hardy-Weinberg equilibrium, which was tested using the chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using multiple logistic regression, which was adjusted for the presence of *H. pylori*. The polymorphism and haplotype analyses were performed using the SNPStats and GraphPad InStat programs. Statistical significance was accepted as $p < 0.05$.

3. Results

Considering all groups of patients (control, gastritis, and cancer) and the polymorphisms of the IL-2 gene (-330 T > G and +114 T > G), only the cancer group was found to be out of Hardy–Weinberg equilibrium in the case of the two polymorphisms. According to the statistical analysis, the −330 T > G and +114 T > G polymorphisms in the control group versus the gastritis group were found to have a linkage disequilibrium (D′ = 0.9062). Meanwhile, the linkage disequilibrium (D’) of the control group versus the cancer group was 0.0554, and the D’ of the gastritis group versus the cancer group was found to be 0.2346). *H. pylori* was detected in 135 samples (45.9%); 39 (28.9%) of the patients with gastric cancer, 86 (63.7%) of the patients with gastritis, and 10 (7.4%) of the patients with normal gastric epithelium.

To analyze the association between the polymorphisms and the groups of patients, four models (codominant, dominant, recessive, and log-additive) were used; all were adjusted for the presence of *H. pylori*. Table 1 and 2 shows that the −330 T > G and +114 T > G genotypes and alleles were found to be associated with an increased risk of gastric cancer, with the exception of the control group and gastritis group, which had no association.

The G/G genotype and the G allele of the IL-2 + 114 T > G polymorphism appeared more frequently and were both associated with an increased risk for developing gastric cancer, though not in the