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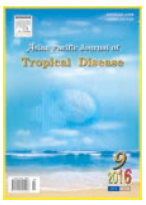

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Association between interleukin-1β polymorphisms and gastric disease in children: A correlation with *Helicobacter pylori*

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ABSTRACT

Objective: To investigate an association between the interleukin-1β (IL-1β)-511 T>C (rs16944), -31 C>T (rs1143627), and/or interleukin-1 receptor antagonist (IL-1RA) polymorphisms and gastritis and then to correlate any associations with the presence of *Helicobacter pylori* (*H. pylori*), *cagA* and *vacA* genes.

Methods: Gastric biopsies were obtained from 377 children with gastric symptoms including 152 males and 225 females aging from 1–15 years with the mean age of (9.41 ± 4.29) years. To characterize the -511 T>C, -31 C>T, and IL-1RA polymorphisms, the PCR-RFLP and PCR-VNTR methods were used. PCR was also used for the diagnosis of *H. pylori* and to determine whether *cagA* and *vacA* genes were present.

Results: The histopathological analysis revealed 206 patients (54.6%) with gastritis and 171 patients (45.4%) with normal gastric tissue. Subjects carrying the -511 T/T genotype were associated with a risk of gastritis (odds ratio (OR) = 2.75, 95% confidence interval (*CI*) 1.45–5.18, $P = 0.0035$). Similar results were found in subjects carrying -31 C/C (OR = 2.27, 95% *CI* 1.13–4.54, $P = 0.0440$). However, the IL-1RA polymorphism did not seem to be associated with gastric disease (OR = 1.38, 95% *CI* 0.58–3.26, $P = 0.2400$).

Conclusions: This data suggests that IL-1β gene cluster polymorphisms and, more specifically, interactions between these polymorphisms and *H. pylori* may be predictors of gastritis risks, which possibly play a relevant role in the susceptibility to or the development of gastric disease early in life.

1. Introduction

Helicobacter pylori (*H. pylori*) is a flagellated, spiral-shaped, microaerophilic Gram-negative bacillus that colonizes the gastric mucosa and is responsible for long-term infection in this membrane. It is the most common cause for chronic gastritis and it is strongly linked to peptic ulcer disease and gastric cancer. However, most people who are *H. pylori* hosts are asymptomatic, and few patients infected with this bacterium develop gastric disease. The wide range of clinical manifestations is associated with various factors, such as environment, the genetic susceptibility of the host and bacterial virulence[1,2].

Chronic *H. pylori* infection induces lesions. These lesions cause chronic inflammation mediated by pro- and anti-inflammatory interleukin, such as interleukin-1β (IL-1β). IL-1β is a cytokine that modulates other inflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL-2, IL-6 and IL-12. These cytokines increase the magnitude of inflammation and may influence the development of gastric disease[3,4].

The IL-1β and IL-1β receptor antagonist proteins belong to the IL-1 gene cluster. This cytokine is a powerful inhibitor of gastric acid secretion, a process which plays a major role in initiating and amplifying the host's inflammatory response to *H. pylori* infection[2]. Caleman Neto *et al.*[5] report that genetic polymorphisms, particularly those that occur in the promoter region, have been associated with an increase in both hypochlorhydria and the synthesis of interleukins and may be considered as a risk factor in the development of gastric disease. Three important polymorphisms are described in the IL-1β gene: IL-1β-511 T>C (rs16944), IL-1β-31 C>T (rs1143627) and interleukin-1 receptor

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The study protocol was performed according to the Helsinki declaration and approve by the Ethics Committee of Marilia Medical School (No. 1119/11), Marilia, São Paulo State, Brazil. Informed written consent was obtained from all of the legal guardians.

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antagonist (IL-1RA)[6-8].

El-Omar *et al.*[6] were the first to report that the IL-1β-511T+/-, -31C+ and IL-1RA 2/2 genotypes were associated with an increased risk of gastric cancer among Scottish and Polish populations. Recently, several studies have tried to associate -511TT and -31CC genotypes with the development of hypochlorhydria and gastric atrophy in response to *H. pylori* infection. According to these studies, these genotypes may increase the production of IL-1β, thus leading to intense inflammation, gastric atrophy, tissue damage, hypochlorhydria and gastric cancer[3,7-9].

Varied frequencies of these polymorphisms across populations, the presence of concomitant *H. pylori* infection, and different genetic pathways for the development of different clinical outcomes are some of the factors that can confuse the true contribution of these polymorphisms to gastric disease[10,11]. Furthermore, the pathogenicity of *H. pylori* increases when the infecting strain expresses the *cagA* gene, which codes for the cagA immunogenic protein. This protein plays a role in the activation of the nuclear factor kappa-B and the production of IL-1 and IL-8. *H. pylori* also expresses the most virulent genotype of vacuolating cytotoxin *vacA*, a gene frequently found in patients with peptic ulcers and gastric cancer[3,12,13].

As mentioned previously, immune response may be considered as a key event in the pathogenic process that leads to gastric disease. Therefore, this study investigated the association between the IL-1β polymorphisms (511 C>T, 31 T>C and IL-1RA) and gastritis and correlated these polymorphisms with the presence of *H. pylori*, the *cagA* gene, and the *vacA* gene in symptomatic children.

2. Materials and methods

2.1. Patient and study protocol

The research was carried out in accordance with the Declaration of Helsinki (revised in 1989) and approved by the Ethics Committee of Marilia Medical School (No. 1119/11), located in Marilia, São Paulo State, Brazil. The research protocol was properly explained to the study population's legal guardians, and all of the legal guardians signed an informed consent form.

The study population consisted of 377 children (152 males and 225 females) aging from 1 to 15 years with a mean age of (9.41 ± 4.29) years. All of the patients had gastric symptoms and received an endoscopy between January 2005 and January 2010 at the Department of Gastroenterology at Marilia Medical School.

Two biopsies were obtained from the antrum of each patient. The first was used for the histology, while the second was used for molecular analysis. The biopsies used in the histological examination were fixed in formalin and were then stained with hematoxylin and eosin and Giemsa. Gastritis was characterized based on the Sydney System. The control group consisted of patients with normal gastric tissue without damage or infiltrated inflammation.

Patients that had undergone treatment via antimicrobial therapy and/or had received treatment via proton pump inhibitors and/or had used non-steroidal antiinflammatory drugs in the three months prior to the endoscopy were excluded from the study.

2.2. DNA extraction and genotyping of Il-1β polymorphisms

DNA was extracted from the gastric biopsies according to the protocol established by the Qiagen Kit QIAamp® DNA Mini Kit (cat No. 51304).

PCR was used for genotyping, followed by an enzyme treatment (restriction fragment length polymorphism) for the -31 T>C and -511 C>T polymorphisms. The IL-1RA gene was analyzed in fragments containing varying numbers of identical tandem repeats with 86 bp tandem repeats, and it was amplified by PCR.

For the characterization of the -31 T>C and -511 C>T polymorphisms, amplification was performed using both the primers and the amplification conditions reported by Caleman Neto *et al.*[5]. Amplified fragments of 240 and 189 bp were used. The amplicons of the -31 T>C and -511 C>T polymorphisms were treated with AluI and AvaI restriction enzymes (Fermentas, USA), respectively.

In the case of IL-1RA, only the PCR was performed, and the PCR products were visualized: amplicons of 410 bp (allele 1, four repeats of the 86 bp region), 240 bp (allele 2, two repeats), 500 bp (allele 3, five repeats), 325 bp (allele 4, three repeats) and 595 bp (allele 5, six repeats)[5].

All fragments were visualized in 2% agarose gel, stained with ethidium bromide and analyzed using an Alpha Imager 2200 (Alpha Innotech Corporation™).

2.3. H. pylori, cagA and vacA detection

H. pylori, *cagA*, and *vacA* detection was performed by PCR. For the diagnosis of *H. pylori*, a 150-bp fragment from the 16s rRNA gene was amplified to evaluate the *cagA* and *vacA* genes, and the protocol described by Pereira *et al.*[14] was used. All fragments were visualized in 2% agarose gel, stained with ethidium bromide and analyzed using an Alpha Imager 2200 (Alpha Innotech Corporation™).

2.4. Statistical analysis

The Hardy–Weinberg equilibrium was analyzed using the *Chi*-square test. Odds ratios (ORs) and 95% confidence intervals (*CI*s) were calculated using multiple logistic regression, which was adjusted for the presence of *H. pylori, cagA*, and *vacA* genes. Linkage disequilibrium between each polymorphism was measured using D'. Statistical analysis was performed in the SNPstats program. Statistical significance was accepted as $P < 0.05$.

3. Results

Genotype and allele frequencies were presented in Table 1. The 1/3 and 1/4 alleles of IL-1RA were excluded from the analysis because of the small sample number with these genotypes. The minor allele frequencies of the -511 C>T, -31 T>C, and IL-1RA polymorphisms were 0.47, 0.47 and 0.22, respectively. When all of the patients were considered, only the -511 C>T polymorphism was found to be within the Hardy-Weinberg equilibrium. According to the SNPstats software, the -511 C>T and -31 T>C polymorphisms were found to be in linkage disequilibrium (D' = 0.7153), as was -31 T>C and IL-1RA (D' = 0.3385), as well as -511 C>T and IL-1RA (D' = 0.3554). *H. pylori* was detected in 102 (27.0%) samples; 86 (84.3%) of these patients had gastritis and 16 (15.7%) had normal gastric epithelium.

To analyze the association between polymorphisms and gastritis, four models (codominant, dominant, recessive, and log-additive) were used. All of them were adjusted for the presence of *H. pylori*. Table 1 shows that -511 C>T and -31 T>C genotypes and alleles were found to be associated with an increased risk of gastritis.

The T/T genotype and the T allele of the IL-1β-511 C>T polymorphism appeared more frequently and were both associated with an increased risk of gastritis. The C/C genotype and the C allele of the IL-1β-31 T>C polymorphism were found to be associated with gastritis as well. The IL-1RA polymorphism, however, did not seem to be associated with gastric disease (Table 1).

Table 1

Genotypes and allele frequencies plus an analysis of the IL-1β polymorphisms in the gastritis group and the control group.

Polymorphism	Genotype/allele	Control [n (%)]	Gastritis [n (%)]	*P*
IL-1β-31 T>C	T/T	48 (0.28)	45 (0.22)	
	T/C	102 (0.60)	109 (0.53)	
	C/C	21 (0.12)	52 (0.25)	
Codominant-OR (95% *CI*)	T/T		1.00	
	T/C		1.15 (0.68–1.94)	0.0440*
	C/C		2.27 (1.13–4.54)	
Dominant-OR (95% *CI*)			1.35 (0.81–2.23)	0.2500
Recessive-OR (95% *CI*)			2.06 (1.14–3.72)	0.0150*
Log-additive-OR (95% *CI*)			1.46 (1.04–2.05)	0.0280*
	T	198 (0.58)	199 (0.48)	
	C	144 (0.42)	213 (0.52)	
OR (95% *CI*)			1.47 (1.10–1.96)	0.0100*
IL-1β-511 C>T	C/C	58 (0.34)	53 (0.26)	
	C/T	88 (0.51)	91 (0.44)	
	T/T	25 (0.15)	62 (0.30)	
Codominant-OR (95% *CI*)	C/C		1.00	
	C/T		1.19 (0.71–1.99)	0.0035*
	T/T		2.75 (1.45–5.18)	
Dominant-OR (95% *CI*)			1.54 (0.95–2.49)	0.0780
Recessive-OR (95% *CI*)			2.46 (1.42–4.27)	0.0010*
Log-additive-OR (95% *CI*)			1.61 (1.18–2.20)	0.0024*
	C	204 (0.60)	197 (0.48)	
	T	138 (0.40)	215 (0.52)	
OR (95% *CI*)			1.61 (1.20–2.15)	0.0008*
IL-1β-RN	1/1	111 (0.65)	121 (0.59)	
	1/2	41 (0.24)	62 (0.30)	
	2/2	11 (0.07)	17 (0.08)	
	1/3	5 (0.02)	5 (0.03)	
	1/4	3 (0.03)	1 (0.01)	
Codominant-OR (95% *CI*)	1/1		1.00	
	1/2		1.52 (0.92–2.52)	0.2400
	2/2		1.38 (0.58–3.26)	
Dominant-OR (95% *CI*)			1.49 (0.93–2.37)	0.0920
Recessive-OR (95% *CI*)			1.21 (0.52–2.82)	0.6600
Log-additive-OR (95% *CI*)			1.31 (0.91–1.87)	0.1400
	1	263 (0.81)	304 (0.76)	
	2	63 (0.19)	96 (0.24)	
OR (95% *CI*)			1.32 (0.92–1.88)	0.0770

OR adjusted for the presence of *H. pylori;* *: Statistically significant.

In the analysis of the correlations between the *cagA* and *vacA* genes, the polymorphism and risk of chronic gastritis, only the *H. pylori*-positive patients were analyzed. Considering these criteria, no significant difference was found when the results were adjusted for the *cagA* and *vacA* genes (data not shown).

4. Discussion

Since the first study by El-Omar *et al.*[6], other studies have been performed to evaluate the association between genetic variations in the IL-1β gene cluster and gastric disease[1,5,10,15]. The results of these studies have been inconsistent. Here, we have performed the first study on Brazilian children that has determined an increased risk of developing gastritis early in life to be associated with polymorphisms of the IL-1β gene and the presence of *H. pylori*. One of the most important points of the present study is the finding that when *H. pylori* is associated with specific IL-1β genotypes, it can be a cause of gastritis with a poor prognosis and possible evolution to gastric cancer.

This evolution was therefore found to involve the biological role of IL-1β, its effects on inflammation and infection situations. The local production of IL-1β modulates the magnitude of the immune/inflammatory response; the IL-1β-31*C and -511*T alleles can affect the transcription level and, in turn, cytokine secretion. This process leads to high-level expression of IL-1β and reduced gastric acid output, both of which facilitate colonization by *H. pylori* bacteria and, as a consequence, pangastritis and atrophic gastritis. Both of these types of gastritis are risk factors for gastric cancer[16].

Recently, Figueiredo *et al.*[1] and Datta De *et al.*[17] reported the location at which *H. pylori* colonies may be shifted from the antrum to the corpus, which depends on gastric acid levels and the inflammatory response. This shift may increase the host's risk of developing atrophic gastritis or gastric cancer.

IL-1β concentration seems to be a determining factor in the etiology and progression of gastric disease. Our hypothesis is enhanced by the findings reported by Chakravorty *et al.*[18] who revealed that *H. pylori*-infected individuals who were also -31T carriers had a 10-fold increase in promoter activity as compared to -31C carriers by using a quantitative analysis of mucosal IL-1β mRNA. These authors suggested that individuals with the -31C/C genotype had significantly lower IL-1β transcript levels, which means that they have less mRNA and secrete less IL-1β and are therefore susceptible to duodenal ulcers.

Sun *et al.*[8] found a significant association between the IL-1β-31 C>T polymorphism and *H. pylori* infection in Asian and Latin American populations, in which carriers of the T allele appeared to be at higher risk of *H. pylori* infection and to exhibit significantly higher levels of IL-1β than those with the C allele.

Following the same reasoning, Erzin *et al.*[19] found that the -31C/C and -511T/T genotypes appeared more frequently among Turkish patients with gastric disease. Similarly, Li *et al.*[20] identified that -511 T/T was a risk factor for gastric cancer in the presence of *H. pylori* infection in a Chinese population.

Another important factor verified in this study is the strong linkage disequilibrium between the -511 and -31 polymorphisms. Previous studies suggest that a strong linkage disequilibrium amplifies the interaction between genomic DNA and transcriptional factors, and that this disequilibrium is therefore associated with increased transcription efficiency[21-23].

El-Omar et al.[24] also showed that the -31 and -511 polymorphisms were in almost complete linkage disequilibrium. They found that the -31C and -511T alleles were associated with an increased risk of gastric disease and its precursors were in response to *H. pylori* infection. In this study, only the IL-1β-511 polymorphism was within the Hardy-Weinberg equilibrium. Despite its association with chronic gastritis, the -31 polymorphismis was not within the Hardy-Weinberg equilibrium. Furthermore, it presents strong linkage disequilibrium with the -511 polymorphism. The Hardy-Weinberg equilibrium of the -31 polymorphism may be explained by the polymorphism's strong linkage disequilibrium with the -511 polymorphism. Therefore, the real risk factor for gastritis is the -511 polymorphism, which is within the Hardy-Weinberg equilibrium and which strongly influences the -31 polymorphism.

In contrast with our results, Santos *et al.*[25] studied Brazilian patients infected with *H. pylori* and found an association between the IL-1β-511 CT/CC genotypes and gastritis as well as between these genotypes and gastric cancer. These results are similar to those previous studies conducted on Mexican patients by Martinez-Carrillo *et al.*[3] and studies conducted in Caucasian and Chinese populations by Yu *et al.*[26].

In the case of the IL-1RA polymorphism, studies suggest that this single nucleotide polymorphism is closely associated with both the regulation of IL-1β activity and gastric cancer development, as reported by Camargo *et al.*[27]. However, our results indicated that there was no association between IL-1RA genotypes and gastric disease in Brazilian children, which is similar to results obtained in studies by Santos *et al.*[25] in their additional study on a Brazilian

population.

Our findings demonstrate that IL-1β gene cluster polymorphisms may play a relevant role in children's susceptibility to gastric disease. Our results also reflect the increased risk of gastritis experienced by *H. pylori*-infected patients with IL-1β-511 T/T and -31 C/C genotypes. Identifying genetic risk predictors would aid in the clinical management of people with chronic infection and would lead to improved diagnosis, risk prediction, and clinical care.

Conflict of interest statement

We declare that we have no conflict of interest.

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