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ORIGINAL

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Interleukin-1β polymorphisms, *Helicobacter pylori* infection in individuals from Northern Brazil with gastric adenocarcinoma

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Abstract Gastric carcinogenesis is a complex, multistep process, which may be influenced by many factors and is the second most common type of malignancy and the second most-common cause of mortality in the word. Interleukin-1 is up-regulated in the presence of *Helicobacter pylori* and is important for initiating and amplifying the inflamatory response to this infection. Recently interleukin-1 polymorphisms have been associated with the development of gastric adenocarcinoma. In this study we investigated the presence of *H. pylori* and host genotypes that are highly associated with gastric alterations. DNA samples were extracted and PCR-RFLP was utilized for genotyping *IL-1B (-511)* polymorphisms, PCR-VNTR was utilized for genotyping *IL-1RN,* and PCR-CTPP was utilized for genotyping *IL-1B (-31)*, the presence of *H. pylori* was detected by the urease test. Our results indicate a correlation between *H. pylori* infection and the development of gastric cancer. We did not find an association between the presence of genotype T (thymine) in bases -511 and -31 and gastric adenocarcinoma. We also did not find any association between this polymorphism and specific type of tumor (diffuse type and intestinal type).

Key words *Helicobacter pylori* • Interleukin-1β polymorphisms • Gastric cancer

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Introduction

Gastric carcinogenesis is a complex, multistep process, which may be influenced by many factors [1]. It is the second most-common type of malignancy and is also the second most-common cause of mortality worldwide [2, 3]. The city of Belém in Pará Brazil is 11th in gastric cancer morbidity per inhabitant among all cities worldwide that have data on cancer incidence. Infections can cause cancer through a variety of mechanisms, including direct cell transformation or immunosuppression yielding reduced cancer immunosurveillance and chronic inflammation [4]. *Helicobacter pylori* infection has been increasingly identified in gastric cancer [5] and The International Agency for Research on Cancer of the World Health Organization classified *H. pylori* as a group I carcinogen [6].

The biological factors that influence clinical outcome in *H. pylori* infection have been extensively studied. In addition to immunological factors in the host, bacterial virulence determinants in *H. pylori* strains are likely to play a crucial role, in the development of gastric cancer [5].

H. pylori infection first induces neutrophilic gastritis, which progresses to active chronic gastritis in most people [6]. Persistent inflammation, possibly intensified via the

inflammatory cytokine cascade and the generation of *H. pylori*-specific T and B cell immune responses [5], eventually leads to gastric atrophy, hypochlorhydria, and increased risk of gastric carcinoma [1, 7–9].

Interleukin-1β (IL-1β) is a potent proinflammatory cytokine that is up-regulated in the presence of *H. pylori* and is important for initiating and amplifying the inflammatory response to this infection [10, 11]. The IL-1 receptor antagonist (IL-1ra) is a naturally occurring anti-inflammatory cytokine that competitively binds to IL-1β [12].

Three diallelic *IL-1β* polymorphisms have been reported, all representing C (cytosine) to T (thymine) base transitions at positions -511, -31, and +3954 base pairs (bp) from the transcriptional start site [13]. There are conflicting data regarding the functional effects of these polymorphisms on IL-1β production [14, 15].

The *IL-RN* gene has a penta-allelic 87-bp tandem repeat (VNTR) in intron 2, of which the less-common allele 2 (IL-1RN*2) is associated with a wide range of chronic inflammatory and autoimmune conditions [16]. El-Omar et al. [13] recently reported that *IL-1β* (encoding IL-1β) and *IL-RN* (encoding IL-1ra) gene polymorphisms (IL-1β -31T, IL-1β -511T, and IL-1RN*2 alleles) are associated with an increased risk of hypochlorhydria in *H. pylori*-infected first-degree relatives of subjects with gastric carcinoma.

In this study we investigated the presence of *H. pylori* and the host genotypes that are most associated with gastric alterations. Our aim was to relate the presence of *H. pylori* in Brazilian patients with gastric cancer and *IL-β* and *IL-RN* polymorphisms.

Materials and methods

Patients with gastric adenocarcinoma and control subjects

A total of 56 subjects diagnosed with gastric adenocarcinoma from the north of Brazil (Pará) were enrolled in this study. The ethnicity, age, and sex of the patients and anatomical sites of the tumors were obtained from medical records or tumor registries. The patients did not have chemotherapy or radiotherapy prior to surgery or any other diagnosed cancer. A genetic study was approved by the ethics committee of the Hospital Universitário João de Barros Barreto (HUJBB) and samples were collected from March 2000 until February 2003.

Study controls were blood donors at the Hemocentro Faculdade de Medicina de Marília-SP, Brazil. We selected a control sample with the same number of male and female individuals as in the patient group as well as with the same ages.

Rapid urease test and histology

One corpus and one antrum sample were placed in a tube containing Christensen's 2% urea agar and examined within 24 h of incubation at 37ºC for urea hydrolysis. Two corpus and two antrum samples were used for histopathology and *H. pylori* detection. Tumors were fixed in formalin, dehydrated in alcohol and xylene, and embedded in paraffin. Sections (5 μm) were obtained and stained with hematoxylin-eosin. Tumors were classified as intestinal and diffuse type according to Laurén [17].

DNA extraction

DNA for polymerase chain reaction (PCR) was extracted directly from one corpus and one antrum biopsy by using the QIAamp tissue kit provided by Qiagem. PCR assays were performed with approximately 100 ng of total DNA.

IL-1β and antagonist receptor genotyping

IL-1β -511 (C-T) restriction fragment length polymorphism
Primers -511a (CTGCATACCGTATGTTCTCTGCC)/-511b (GGAATCTTCCCACTTACAGATGG) were used to amplify the region containing the AvaI polymorphic site within exon 5 of the *IL-1β* gene [18]. Amplification was performed at 94ºC for 5 min; 30 cycles at 94ºC for 30 s, 60ºC for 30 s, and 72ºC for 30 s, followed by one cycle at 72ºC for 5 min and cooling at 4ºC. The PCR products were digested with AvaI at 37ºC overnight. Fragments were separated by electrophoresis in 4% agarose GTG gels and stained with ethidium bromide. A C/C allele was identified if two bands of 80 and 109 bp were obtained, a T/T allele was identified if a single band of 189 bp was obtained, while allele T/C was identified if bands of 80, 109, and 189 bp were observed (Fig. 1).

IL-RN (IL-Ra) Variable number of tandem repeat polymorphisms
Fragments containing variable numbers of identical tandem repeat of 87 bp were amplified using the primers flanking the region, IL-RNa (TCCTGGTCTGCAGGTAA) and IL-RNb (CTCAGCAACACTCCTAT) [19]. Amplification was performed at 94ºC for 5 min, 40 cycles at 94ºC for 30 s, 60ºC for 30 s, and 72ºC for 30 s, followed by one cycle at 72ºC for 5 min and cooling at 4ºC. PCR products were 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) (Fig. 2A).

IL-1β -31(T-C) PCR confronting two-pair primers
Amplification was performed using primers described by Hamajima et al 2000 [18] under the following conditions: 94ºC for 5 min; 40 cycles at 94ºC for 1 min, 53ºC for 1 min, and 72ºC for 1 min, followed by one cycle at 72ºC for 5 min and cooling at 4ºC. We found PCR products of 121 bp and 238 bp for the TT allele, 121 bp, 155 bp and 238 bp for the TC allele and 155 bp and 238 bp for the CC allele (Fig. 2b). PCR products of -31 base transitions and *IL-RN* were analyzed by electrophoresis in 2% agarose gels stained with ethidium bromide.

Statistical analysis

Statistical analysis involved Fisher's exact test, chi-squared test, and the binomial proportion.

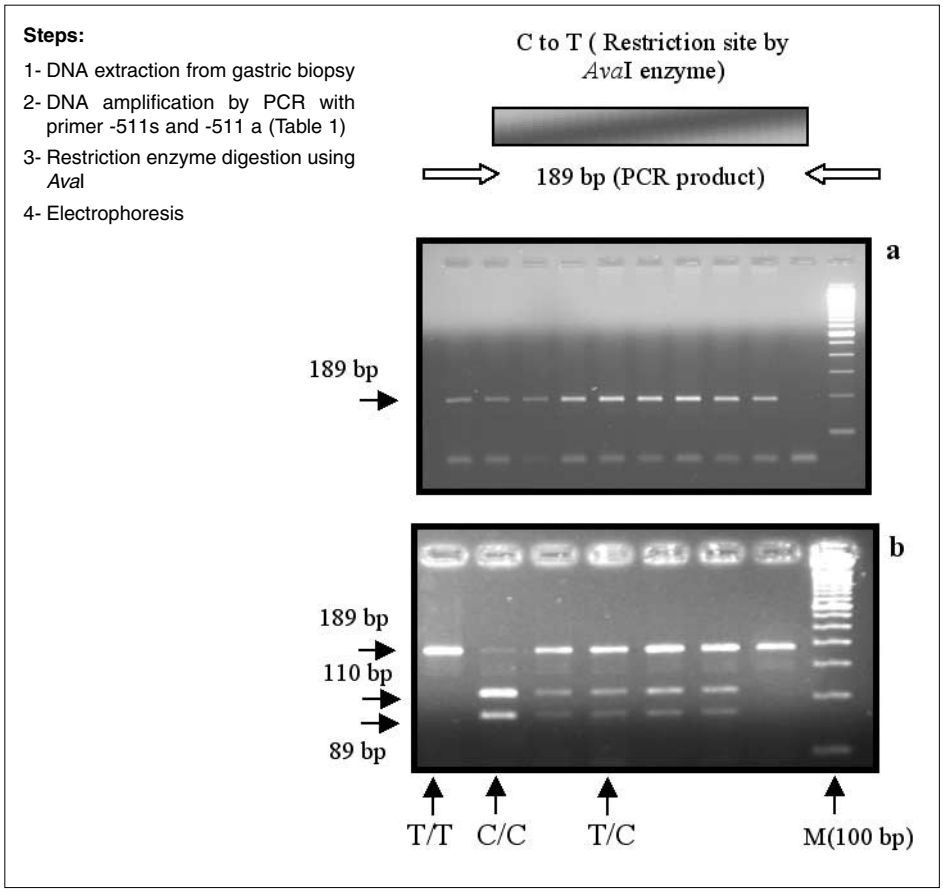

Fig. 1 Detection of interleukin-1 (*IL-1*) β C to T transition at -511 by polymerase chain reaction (*PCR*)-restriction fragment length polymorphism. C/C (homozygote C allele – 89 and 110 bp), C/T (heterozygote – 89, 110, and 189 bp) and T/T (homozygote T allele – 189 bp). **a** Agarose gel showing *IL-1β* -511, PCR product of 189 bp. **b** 4% agarose GTG gel showing *IL-1β* -511 after digestion with *Ava*I enzyme. [*M* marker ladder 100 bp (GIBCO)]

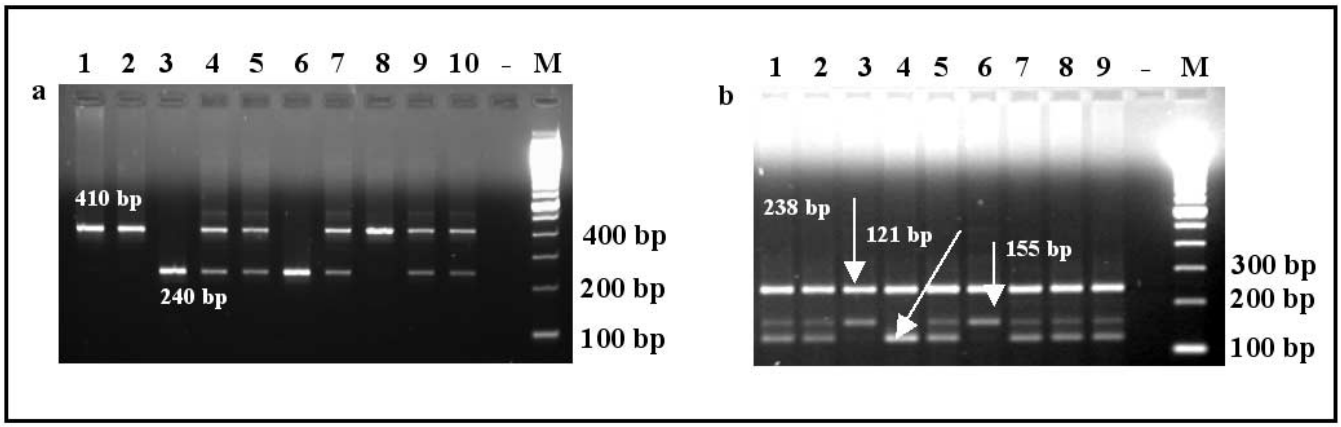

Fig. 2a Agarose gel showing *IL-RN* alleles. Lanes 1, 2, and 8 allele 1/1 (410 bp, four repeats), lanes 3 and 6 allele 2/2 (240 bp, two repeats), lanes 4, 5, 7, 9 and 10 allele 1/2 (240 and 410 bp, two repeats and four repeats/heterozygous), (-) negative control. **b** Agarose gel showing *IL-1β -31*, PCR-CTPP. Lanes 1, 2, 5, 7, 8 and 9 heterozygous allele T/C (121, 155, and 238 bp), lanes 3 and 6 homozygous, allele CC (155 and 238 bp)

Results

A total of 56 gastric cancer (32 diffuse type and 24 intestinal type) and 56 control subjects were included in this study. Data of *H. pylori*-positive gastric carcinoma patients are listed in Table 1. Of the 56 subjects studied, 42 (75%) were urease test positive for *H. pylori* infection, showing an association between the infection and the presence of gastric adenocarcinoma. Of the 56 subjects, 42 were male and 14 were female, with no association between male sex and gastric adenocarcinoma (λ^2=0.3).The proportion of *H. pylori* positive men and women did not differ significantly. The average age of gas-

tric carcinoma patients was 56 years. No age-related differences were observed.

The genotypes of the -511, -31 *IL-1β* and *IL-RN* genetic polymorphisms are summarized in Tables 2 and 3. The genotype frequency of T (thymine) in *IL-1β* -31 was 69% and *IL-1β*-511 was 86%. These frequencies are a result of combining homozygotes and heterozygotes of thymine nucleotide. We found no statistical significant difference between the IL polymorphisms and sex (P=1).

Only 4 (11%) tested gastric adenocarcinoma cancer patients had *IL-RN**2/*2 genotypes. The frequency of *IL-1RN**2/*2 (allele 2 in homozygote) genotype was not associated with an increased risk of either gastric adenocarcinoma.

Table 2 shows the IL-1β genotype of male and female patients with and without *H. pylori* infection and controls (blood donors). There was no association between any group and *IL-1β* polymorphisms.

T and C carrier male individuals did not differ in relation to the presence or absence of *H. pylori* infection (P<0.05); the same was true for T and C carrier females (P<0.05).

In cancer patients (56 subjects) the genotype distributions of *IL-1β* -31 polymorphisms as well as -511 polymorphisms are within Hardy-Weinberg equilibrium (P>0.05). We did not find any association between the frequencies of *IL-1β* -511, -31, and *IL-1RN* with histological type of gastric adenocarcinoma (Table 3).

Table 1 Characteristics of gastric carcinoma patients with and without *Helicobacter pylori* infection

Urease test diagnostic	Total/diagnostic	Male sex	Female Sex
H. pylori positive	37 (66%)	27 (64%)	10 (71%)
H. pylori negative	19 (34%)	15 (36%)	4 (29%)
Total/sex	–	42	14

Table 2 Distribuition of genotypes of *IL-1β* and *IL-RN* according to sex and urease test for positive and negative *H. pylori* status and negative control group (blood donors) (*VNTR* variable number of tandem repeat polymorphisms)

	Males (n=84)						Females (n=28)					
	Hp-	(%)	*Hp+*	(%)	Control	(%)	*Hp-*	(%)	*Hp+*	(%)	Control	(%)
IL-1β -31 (C to T)												
C/C	03	20	10	37	09	21	02	50	02	20	01	07
C/T	08	53	12	44	23	55	01	25	04	40	11	78
T/T	04	27	05	19	10	24	01	25	04	40	02	15
T carrier	12	80	17	63	33	78	02	50	08	80	13	93
C carrier	11	73	22	81	32	76	03	75	06	60	12	86
Total	15		27		42		04		10		14	
IL-1β -511 (C to T)												
C/C	04	27	02	07	03	07	01	25	04	40	01	07
C/T	08	53	14	52	28	67	02	50	03	30	12	86
T/T	03	20	11	41	11	26	01	25	03	30	01	07
T carrier	11	73	25	92	39	93	03	75	06	60	13	93
C carrier	12	80	16	59	31	74	03	75	07	70	13	93
Total	15		27		42		04		10		14	
IL-RN (86 bp VNTR at intron 2)												
1/1	04	27	10	37	20	48	01	25	04	40	09	64
1/2	10	67	13	48	20	48	03	75	06	60	04	28
1/4	–	–	01	04	–	–	–	–	–	–	–	–
2/2	01	06	03	11	02	4	–	–	–	–	01	08
Total	15		27		42		04		10		14	

Table 3 Comparison of *IL-1β* -511, -31 and *IL-RN* genotype frequencies in control (blood donors) and gastric carcinoma patients according to the histologic type of the tumors

	Gastric carcinoma			
	Intestinal	(%)	Diffuse	(%)
IL-1β (-511)				
T/T	06	25	13	40
T/C	16	67	10	30
C/C	02	08	09	30
T carriers	18	67	23	72
Total	24		32	
IL-1β (-31)				
T/T	03	12	11	34
T/C	14	58	11	34
C/C	07	30	10	32
T carriers	21	87	22	69
Total	24		32	
IL-RN				
1/1	07	29	12	37
1/2	17	71	15	47
1/4	–	–	01	03
2/2	–	–	04	13
Total	24		32	

Discussion

In this study we assessed the association between the *IL-1β* -511T, -31T, and *IL-1RN* variable number of tandem repeat polymorphisms with gastric adenocarcinoma in individuals from Northern Brazil. *H. pylori* infection first appears as neutrophilic gastritis, which progresses to active chronic gastritis in most people [6]. Recruitment and activation of immune cells in the underlying mucosa involves, among other molecules, proinflammatory cytokines such as IL-1β and tumor necrosis factor-α as part of non-specific immunity [20].

The complex role of cytokines in these inflammatory responses includes counter-regulatory elements, such as IL-1ra, which act to down-regulate inflammation [21]. Recently, *IL-1β* has been reported to be an important proinflammatory cytokine that is highly expressed in the gastric mucosa of *H. pylori*-positive hosts; gastric acid secretion is low in this population [14, 22].

El-Omar et al. [13] examined the association of particular genotypes with a low acid state in Scottish and Polish gastric cancer patients and control. They suggest that individuals with the -31TT or TC and -511TT or TC genotypes overexpress gastric *IL-1β* in response to *H. pylori* infection, leading to increased inflammation and lower stomach acidity [23].

Machado et al. [21] found evidence of interaction between -511T and *IL-RN**2 in the modulation of the inflammatory response [24]. The proinflammatory effect of these alleles might be explained by their DNA sequences. *IL-1β* -31 and -511 T are a TATA-box polymorphism that markedly affects in vitro DNA-protein interactions, hence controlling the expression of IL-1β [23].

This study showed no association between *IL-1β* -511 T genotype (homozygous or heterozygous) and gastric adenocarcinoma patients and controls. A Japanese study showed that the -31 TT genotype has a higher risk for *H. pylori* infection and gastric adenocarcinoma [25], results at variance with our study. The association between the *IL-1β* -511T allele and diffuse and intestinal type carcinoma did not reach statistical significance.

IL-1RN 2 has been associated with gastric cancer risk [26]. However in the Japanese population this association is not observed [27]. Our results show that only 11% of gastric adenocarcinoma patients had a *IL-1RN* *2 genotype, results that are similar to Korean populations [13].

In summary, our results do not confirm that *IL-1β* polymorphisms that enhance IL-1β expression and are associated with increased risk of gastric adenocarcinoma. There was no association with the *IL-1RN** genotype. This study is important because the effects of IL-1β polymorphisms are still unclear in between different populations. Further investigations are required in different ethnic populations.

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