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Tese

# Fatores de risco associados à infecção pelo *Helicobacter pylori* e ao desfecho clínico

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Pelotas, 2014

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# Fatores de risco associados à infecção pelo *Helicobacter pylori* e ao desfecho clínico

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Para os meus pais, Euzébio e Silvia, com carinho e gratidão.

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“Os sonhos são como uma bússola, indicando os caminhos que seguiremos e as metas que queremos alcançar. São eles que nos impulsionam, nos fortalecem e nos permitem crescer”.

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## Resumo

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*Helicobacter pylori* é uma bactéria que infecta a mucosa gástrica de aproximadamente 50% da população humana mundial. A infecção por *H. pylori* tem sido associada a uma variedade de doenças, tais como gastrite, doença ulcerosa péptica e carcinoma gástrico. Acredita-se que a combinação de variáveis ambientais/comportamentais, fatores genéticos do hospedeiro e genes de patogenicidade bacteriana possa interferir na gravidade da lesão gástrica e no desfecho clínico de infecção por *H. pylori.* Desta forma, o presente trabalho objetivou determinar a frequência e potenciais fatores de risco da infecção por *H. pylori*, assim como identificar os polimorfismos nos genes da *interleucina (IL)-1, IL-6, IL-8* e *IL-10* e suas associações com infecção por *H. pylori,* com *cytotoxin associated gene A* (gene *cagA)* de *H. pylori* e com patologias gástricas. Para isso, foi conduzido um estudo transversal, incluindo amostras de biópsia gástrica de 227 pacientes, submetidos à endoscopia digestiva alta. Um questionário foi aplicado aos pacientes antes da endoscopia. O diagnóstico de *H. pylori* baseou-se na histologia e na *polymerase chain reaction* (PCR). O gene *cagA* foi identificado por PCR. Os polimorfismos nos genes da *IL-1B* (nas posições -511, -31 e +3954), *IL-6* (na posição -174), *IL-8* (na posição -251) e *IL-10* (nas posições -819 e -592) foram detectados por PCR-*restriction fragment length polymorphism* (RFLP). A análise do polimorfismo *variable number of tandem repeat* (VNTR) no gene *IL-1RN* foi realizada por PCR, seguida por eletroforese em gel de agarose. Dos 227 pacientes incluídos neste estudo, 151 foram positivos para *H. pylori* e 76 negativos. Com base nos questionários aplicados aos pacientes, verificou-se existir associação significativa entre a presença de *H. pylori* e a aglomeração familiar, a idade, e os diagnósticos endoscópicos e histológicos. Quanto aos fatores genéticos do hospedeiro observou-se que os polimorfismos nas regiões promotoras dos genes *IL-1B* e *IL-8* estavam significativamente relacionados à infecção por *H. pylori.* As cepas de pacientes *H. pylori* positivos portadores do gene *cagA* foram significativamente associadas com o polimorfismo da *IL-1B* na posição -511. Adicionalmente, na presença da infecção pelo *H. pylori,* demonstrou-se que os polimorfismos na região promotora do gene *IL-1B* estiveram correlacionados com um risco aumentado de gastrite, enquanto aqueles nos genes da *IL-8* e da *IL-10* foram associados a um risco elevado da doença ulcerosa péptica. Conclui-se que, provavelmente, os polimorfismos genéticos do hospedeiro exerçam influência sobre o curso e a gravidade das doenças gástricas. Espera-se que os resultados deste trabalho possam contribuir, em breve, tanto para a prevenção dessas desordens quanto para uma terapia mais adequada e eficiente, isto porque o conhecimento das bases moleculares do hospedeiro e do microrganismo viabiliza o desenvolvimento de testes moleculares, os quais poderão ser aplicados em prol de um correto prognóstico das patologias gástricas.



**Palavras-chave**: Desordem gástrica, Epidemiologia, Gene de patogenicidade bacteriana, Polimorfismo genético humano.

## Abstract

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*Helicobacter pylori* is a bacterium that infects the gastric mucosa of approximately 50% of the world´s human population. *H. pylori* infection has been associated with a variety of diseases such as gastritis, peptic ulcer disease and gastric carcinoma. It is believed that the combination of environmental/behavioral variables, host genetic factors and bacterial pathogenicity genes can interfere in the severity of gastric damage and in the clinical outcome of *H. pylori* infection. In this sense, the present study aimed to determine the frequency and potential risk factors of the *H. pylori* infection, even as identify polymorphisms in the *interleukin (IL)-1, IL-6, IL-8* and *IL-10* genes and their associations with *H. pylori* infection, with *cagA* gene of *H. pylori,* and with gastric pathologies. For this, it was conducted a cross-sectional study, including gastric biopsy samples from 227 patients, submitted to upper gastrointestinal endoscopy. A questionnaire was applied to the patients before endoscopy. The diagnosis of *H. pylori* was based in histology and in polymerase chain reaction (PCR). The *cagA* gene was identified by PCR. The polymorphisms in the genes of *IL-1B* (at positions -511, -31 and +3954), *IL-6* (at position -174)*, IL-8* (at position -251) and *IL-10* (at positions -819 and -592) were detected by PCR-restriction fragment length polymorphism (PCR-RFLP). The analysis of the variable number of tandem repeat (VNTR) polymorphism in the *IL-1RN* gene was performed by PCR followed by electrophoresis agarose gel. Of the 227 patients included in this study, 151 were positive for *H. pylori* and 76 negative. Based on the questionnaires applied to the patients, it was found a significant association between the presence of *H. pylori* and the household crowding, the age, and the diagnoses histological and endoscopic. As for host genetic factors was observed that polymorphisms in the promoter region of the *IL-1B* and *IL-8* genes were significantly related with *H. pylori* infection. The strains from patients *H. pylori* positive carrying the *cagA* gene were significantly associated with the polymorphism of *IL-1B* at position -511. Additionally, in the presence of *H. pylori* infection, it was demonstrated that polymorphisms in the promoter region of the *IL-1B* gene were correlated with an enhanced risk of gastritis, while those in the *IL-8* and *IL-10* genes were associated with higher risk of peptic ulcer disease. We conclude that probably, the host genetic polymorphisms exert influence in the course and gravity of gastric diseases. It is hoped that the results of this work may contribute soon, to the prevention of these diseases and for a more appropriate and effective therapy, this because the knowledge of the molecular basis of the host and the microorganism enables the development of molecular tests that may to be applied toward a correct prognosis of gastric pathologies.



**Keywords:** Gastric disorder*,* Epidemiology*,* Bacterial pathogenicity gene, Human genetic polymorphism.

## Lista de Abreviaturas

*cagA* gene - *cytotoxin associated gene A* (*citotoxina associada ao gene A*)

IL – interleucina

OMS – Organização Mundial da Saúde

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## 1 INTRODUÇÃO GERAL

A bactéria *Helicobacter pylori* foi descoberta no ano de 1983, na Austrália, pelos pesquisadores Robin Warren e Barry Marshall (Warren; Marshall, 1983). Posteriormente, em 1994, a Organização Mundial da Saúde (OMS) classificou o *H. pylori* como carcinógeno para humanos (IARC, 1994). O reconhecimento pela identificação da bactéria e sua associação com a doença gástrica levou, no ano de 2005, a concessão do Prêmio Nobel de Medicina para Warren e Marshall (Wroblewski et al., 2010).

O *H. pylori* infecta cerca de 50% da população humana mundial. A desordem primária, a qual ocorre após a infecção com o microrganismo, é conhecida como gastrite crônica e consiste na inflamação da mucosa gástrica. As eventuais complicações desta inflamação crônica que afeta a fisiologia gástrica, denominam-se doença ulcerosa péptica e carcinoma gástrico (Kusters et al., 2006; Wroblewski et al., 2010; Malfertheiner, 2011). Estima-se que, dentre os indivíduos infectados, 15 a 20% irão desenvolver doença ulcerosa péptica e 1%, aproximadamente, irão desenvolver carcinoma gástrico (Menon et al., 2011).

O presente trabalho partiu do pressuposto de que a gravidade da lesão gástrica e o risco de desenvolvimento dessas desordens no que tange a infecção pelo *H. pylori* parecem ser determinados por fatores da bactéria, por variáveis ambientais/comportamentais e por polimorfismos genéticos do hospedeiro que influenciam nos níveis de secreção de citocinas inflamatórias (Moorchung et al., 2007; Izzotti et al., 2009; Puneet et al., 2012). Sendo assim, este estudo objetivou determinar a frequência e potenciais fatores de risco da infecção pelo *H. pylori*, bem como identificar os polimorfismos nos genes das *interleucinas (ILs)-1, -6, -8* e *-10*, e suas associações com o processo infeccioso causado pela bactéria.

Diante deste contexto, entre março de 2011 e julho de 2014, foram realizados diversos estudos, cujos resultados vieram a ser registrados por meio de quatro manuscritos científicos.

O primeiro trabalho, intitulado *"Helicobacter pylori pathogenicity genes, cytokine polymorphisms and environmental factors affect the development of gastric diseases: an overview",* foi submetido para a Revista da Sociedade Brasileira de

Medicina Tropical e consiste em uma revisão bibliográfica sobre os fatores do ambiente, da bactéria e do hospedeiro que influenciam na susceptibilidade ao desenvolvimento de severas desordens gastroduodenais relacionadas à infecção pelo *H. pylori.*

O segundo manuscrito, denominado *"Helicobacter pylori infection and associated factors"*, aborda a frequência da bactéria *H. pylori* em pacientes da região Sul do Brasil e avalia fatores de risco, como idade e aglomeração familiar, associados à infecção pelo *H. pylori*. Este manuscrito está sob análise da Revista Biomédica do Instituto Nacional de Salud de Colombia.

O terceiro trabalho descreve a frequência dos polimorfismos nos genes da *IL-1B* e da *IL-1RN* e investiga a relação desses polimorfismos com a infecção pelo *H. pylori,* com a *citotoxina associada ao gene A* (gene *cagA)* de *H. pylori* e com o risco de desenvolvimento de gastrite, de doença ulcerosa péptica e de carcinoma gástrico, sendo o mesmo intitulado *"Relationship of interleukin-1B gene promoter region polymorphism with Helicobacter pylori infection and gastritis".* Este trabalho foi submetido ao Journal of Infection in Developing Countries.

Posteriormente, o quarto manuscrito, intitulado *"Gene polymorphism of IL-6, IL-8 and IL-10 and the risk of gastric pathologies in patients infected with Helicobacter pylori",* relata a frequência dos polimorfismos nos genes da *IL-6*, da *IL-8* e da *IL-10* e avalia a associação desses polimorfismos com a infecção pelo *H. pylori* e com o risco aumentado de desenvolvimento de desordens gástricas de maior gravidade. Este manuscrito está sob a avaliação do Journal of Microbiology, Immunology and Infection.

Por fim, é importante registrar que os resultados descritos nos quatro trabalhos supramencionados, que compõem a presente tese, não podem ser analisados isoladamente, nem ensejam o final das pesquisas sobre o assunto, ao contrário, visam estimular e agregar novos dados para as pesquisas científicas presentes e futuras.

# 2 HIPÓTESE E OBJETIVOS

## 2.1 Hipótese

A gravidade do dano gástrico e a diversidade de manifestações clínicas relacionadas à infecção pelo *H. pylori* podem ser influenciadas tanto por variáveis ambientais/comportamentais, quanto por fatores genéticos do hospedeiro e por genes de patogenicidade bacteriana.

## 2.2 Objetivo Geral

Identificar possíveis variáveis ambientais/comportamentais, polimorfismos genéticos do hospedeiro e genes de patogenicidade bacteriana associados com infecção pelo *H. pylori* e com desfechos clínicos de maior gravidade.

## 2.3 Objetivos Específicos

- Fornecer uma visão geral dos fatores ambientais/comportamentais, bacterianos e do hospedeiro que podem influenciar a susceptibilidade a desfechos clínicos severos de infecção por *H. pylori a*través de uma revisão do estado-da-arte*;*
- Detectar o *H. pylori* nas amostras clínicas estudadas*;*
- Verificar se existe relação entre infecção pelo *H. pylori* e idade, gênero, estado civil, escolaridade, fumo, consumo de álcool, aglomeração familiar, tipo de água consumida, renda familiar mensal, tipo de sanitário utilizado, diagnóstico endoscópico e diagnóstico histológico;
- Determinar a frequência dos polimorfismos nos genes que codificam as ILs-1, -6, -8 e -10;
- Avaliar a associação entre a infecção pelo *H. pylori* e a presença dos polimorfismos genéticos nas ILs-1, -6, -8 e -10;
- Investigar a relação entre a presença dos polimorfismos nos genes das ILs-1, -6, -8 e -10 e o diagnóstico endoscópico de mucosa gástrica normal, gastrite, doença ulcerosa péptica e carcinoma gástrico;
- Analisar a associação entre a presença dos polimorfismos genéticos na IL-1, e o gene *cagA* de *H. pylori.*

# 3 CAPÍTULOS

## 3.1 Manuscrito 1 - *Helicobacter pylori* pathogenicity genes, cytokine polymorphisms and environmental factors affect the development of gastric diseases: an overview.

Manuscrito submetido à Revista da Sociedade Brasileira de Medicina Tropical.

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## ABSTRACT

*Helicobacter pylori* is a Gram-negative bacterium that colonizes the stomachs of approximately 50% of the world´s human population. This microorganism is the major causal agent of gastritis and is an important risk factor for the development of peptic ulcer disease and gastric carcinoma. The factors that determine this diversity of clinical outcomes are subject to continuous investigations and are thought to be determined by interaction of bacterial factors, the host immune system and environmental/behavioral variables. *H. pylori* possesses a set of pathogenicity factors, including *cagA, vacA*, *iceA,* and *babA* that are necessary for colonizing the gastric mucosa and establishing a chronic infection. These bacterial factors are essential players in modulating the immune response involved in the initiation of carcinogenesis in the stomach. Host genetic factors contribute to regulation of the inflammatory response and aggravation of mucosal damage once gastric infection with *H. pylori* induces mucosal production of various pro- and anti-inflammatory cytokines in the host. The harmful role of environmental/behavioral factors is related to poor socioeconomic conditions, salt intake, smoking and alcohol consumption. The aim of this review is to provide an overview of bacterial, host and environmental/behavioral factors that influence susceptibility to severe outcomes of *H. pylori* infection. By deciphering the deterministic rules of this interplay, we will eventually be able to predict, treat, and ultimately prevent serious gastroduodenal diseases.

**Keywords:** *Helicobacter pylori.* Pathogenicity Genes. Host Genetic Polymorphism. Gastroduodenal Diseases. Epidemiology.

## INTRODUCTION

*Helicobacter pylori* is a micro-aerophilic, spiral-shaped, flagellar, Gram-negative bacterium that colonizes the gastric mucosa[1,2]. The prevalence of *H. pylori* infection in developing countries is estimated to be between 60–90%. The prevalence in the developed world has steadily declined over the past decades, but is still at levels of 25–35% in many populations[3].

*H. pylori* infection induces chronic gastric inflammation, which is asymptomatic in the majority of the patients. However, it is estimated that 15 to 20% of *H. pylori* infected individuals will develop peptic ulcer disease (PUD) and approximately 1% will develop gastric carcinoma (GC)[4-6]. In 1994, the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) classified *H. pylori* as a carcinogen (group I) for humans[7]. GC is the fourth most common malignancy and the second leading cause of death due to cancer worldwide[8,9]. The interaction of bacterial pathogenicity factors, host aspects and environmental/behavioral factors determine the severity of gastric damage and the clinical outcome of *H. pylori* infection[10].

The *H. pylori* genome shows high plasticity, and genomic variants may be responsible for inducing various types of gastric lesions in the host[11,12]. Several biomarkers of *H. pylori* pathogenicity have been described, including *cagA (cytotoxin-associated gene A)*, *vacA* (vacuolating cytotoxin), *iceA* (induced by contact with epithelium), and *babA* (blood-group antigen-binding adhesin A)[13-16].

The host immune system plays an important role in the pathogenesis of gastroduodenal disorders by regulating the nature and intensity of the inflammatory response to *H. pylori* infection[17]. The inflammatory cells that are recruited to the

gastric mucosa during infection produce several pro- and anti-inflammatory cytokines[18]. In this sense, it is noteworthy that polymorphisms in the genes encoding interleukins (IL) such as IL-1, IL-6, IL-8 and IL-10 can be associated with increased susceptibility to or protection against gastric diseases[19-24].

The combined presence of environmental/behavioral factors, such as household crowding, inadequate eating habits, low socioeconomic conditions, poor hygiene, alcohol consumption and smoking are related to *H. pylori* infection. Additionally, factors such as smoking, eating habits and alcohol consumption have been associated with a higher risk of development of PUD and GC in patients infected with *H. pylori*[25-27].

The aim of this review is to provide an overview of bacterial, environmental/behavioral, and host factors that can influence susceptibility to severe outcomes of *H. pylori* infection. By deciphering the deterministic rules – if any – of this interplay, we will eventually be able to predict, treat, and ultimately prevent serious gastroduodenal diseases.

## *Helicobacter pylori* INFECTION: FROM GASTRIC INFLAMMATION TO GASTRIC CARCINOGENESIS

The infection of the stomach by *H. pylori* induces inflammation of the gastric mucosa (gastritis) and thus profoundly affects gastric physiology[28,29]. There are two distinct types of gastritis, antral-predominant gastritis and pangastritis, which affect not only the antrum but also the corpus[30]. *H. pylori* infection may be associated with decreased or increased gastric acid secretion, depending on anatomic distribution and severity of gastritis. The effect of *H. pylori* on gastric acid secretion is exerted

through various mechanisms, including production of fatty acids, release of inhibitory cytokines, ammonia production, impairment of the feedback between gastrin and acid secretion and induction of damage to gastric-body mucosa[10].

The interplay of gastritis and acid secretion are key determinants in disease outcomes such as PUD and/or GC[28]. Ulceration corresponds to the loss of mucosal integrity as a result of disequilibrium between defensive mucosa-protective factors and aggressive injurious factors. When ulcers develop in the acid-peptic environment of the gastroduodenum, they are called PUD[31]. The development of duodenal ulcers is associated with antral predominant gastritis and increased gastric acid production, whereas the development of gastric ulcers is related to corpus predominant gastritis or pangastritis and reduced gastric acid production. These conditions predispose patients to the development of gastric atrophy (defined as the loss of gastric glands), intestinal metaplasia (defined as the replacement of glandular and/or foveolar epithelium by intestinal epithelium) and GC[32-34].

*H. pylori* infection can induce direct changes on host DNA, such as oxidative damage, methylation, chromosomal instability, microsatellite instability and gene mutations. Interestingly, *H. pylori* infection generates genetic instability in nuclear and mitochondrial DNA and DNA repair deficiency in host cells. *H. pylori* infection promotes gastric carcinogenesis by at least five mechanisms: (1) a combination of increased endogenous DNA damage and decreased repair activities; (2) induction of mutations in the mitochondrial DNA; (3) generation of a transient mutator phenotype that induces mutations in the nuclear genome; (4) disruption of the balance between cell proliferation and apoptosis during *H. pylori* infection; and (5) induction of an intense gastric inflammatory response that lasts decades and produces chronic

oxidative stress and adaptive changes in the gastric epithelial and immune cell pathobiology[35-38].

## ROLE OF BACTERIAL PATHOGENICITY GENES IN GASTRIC DISORDERS

*H. pylori* strains are highly diverse. Therefore, genotyping of the microorganism appears to be clinically relevant and can contribute to the prognosis of infection by this bacterium[39]. The first pathogenicity gene identified in *H. pylori* was *cagA*[13]. This gene is located at one end of a 40-kb DNA insertion called the *cag* pathogenicity island (*cag*PAI). The *cagA* gene product, CagA protein, is secreted through a type IV secretion system (T4SS) and then transported to host cells[40]. Once inside gastric epithelial cells, the CagA undergoes tyrosine phosphorylation in its repeat region motif of five amino acids Glu-Pro-Ile-Tyr-Ala (glutamic acid-proline-isoleucine-tyrosine-alanine/EPIYA). This phosphorylation is mediated by Src kinase. Phosphorylated CagA subsequently binds to Src homology 2 (SH2) domains contained in a number of host cell proteins, including tyrosine phosphatase SHP-2, C-terminal Src kinase (CSK) and adapter protein Crk. This interaction results in cytoskeletal reorganization and cell elongation, a phenotype that leads to the dispersion of cells and morphological changes for "hummingbird phenotype"[41].

SHP-2 has been identified as a key component in various oncogenic signaling pathways[42]. The CagA-SHP-2 interaction constitutes the biological basis of *cagA* as a pathogenicity factor of *H. pylori*. Induction of abnormal proliferation and movement of gastric epithelial cells may result in cellular changes, eventually leading to gastric atrophy and GC[43].

CagA acts as a highly immunogenic antigen. The structure of the *cagA* gene shows a highly conserved 5' region and a 3' region containing a variable number of repeat sequences, leading to variation in the length of the protein and resulting in diverse host responses, including different degrees of inflammatory responses[12]. Variations in the repeat region of the CagA EPIYA motif have also been associated with *H. pylori* pathogenicity. Four distinct types of EPIYA have been identified and classified: EPIYA-A, -B, -C and -D. CSK specifically binds to the tyrosine-phosphorylated EPIYA-A or –B forms, whereas SHP-2 specifically binds to the tyrosine-phosphorylated EPIYA-C or –D forms[41]. *cagA* strains of *H. pylori* found in the Western world typically contain EPIYA-A, -B and -C in the EPIYA repetition region in the C-terminus. In contrast, *cagA* strains from East Asian contain EPIYA-A, -B and a specific sequence of Asian CagA EPIYA-D[44]. The number of segments of EPIYA-C in *H. pylori* strains from Western countries influence the degree of pathogenicity as well as the oncogenic potential, and may serve as a marker for identifying populations at high risk for GC mortality[45,46]. This occurs because the presence of EPIYA-C segments of CagA appear to significantly contribute to transcriptional activation of IL-8 through activation of nuclear factor kappa B (NF-kB)[47]. IL-8 plays a crucial role in chemoattracting and activating neutrophils at the site of infected gastric mucosa[48]. Batista et al.[49] showed that increasing the number of EPIYA-C segments was associated with precancerous gastric lesions and decreased serum levels of pepsinogen I, which reflects the functional and morphological status of the gastric mucosa. Other studies found that the presence of a higher number of CagA EPIYA-C segments is a risk factor for PUD[50,51].

Additional important disease-associated pathogenicity genes of *H. pylori* include *vacA*, *iceA* and *babA*[14-16]. The *vacA* gene encodes a vacuolating cytotoxin

(VacA) that can damage gastric epithelial cells by inducing the formation of cytoplasmic vacuoles[52,53]. The gene consists of three variable regions: the signal region (*s* - encoding the signal peptide) with two alleles, *s1* (subtypes *s1a, s1b, s1c*) and *s2;* the medium region (*m*) with alleles *m1* and *m2*; and the intermediate region (*i*) containing the alleles *i1* and *i2*[53,54]. The combination of alleles of the signal, middle and intermediate regions determines vacuolating cytotoxin production and is associated with the pathogenicity of the bacterium. In general, strains containing the *s1/m1/i1* alleles produce large amounts of vacuolating cytotoxin, which is one reason why this genotype seems to be associated with more severe pathologies such as PUD and GC. In contrast, strains carrying the *s1/m2* and *s2/m2/i2* alleles have moderate and no cytotoxic activity, respectively[14,54-56]. The strains carrying these genotypes are considered less toxic because of their inability to form vacuoles[57]. A recent study showed that the best markers of GC and duodenal ulceration were the vacA *s1* and *i1* genotypes and that the *s* and *i* regions were the key determinants of vacuolating cytotoxin activity[58]. Another study found that strains that carried the *vacA s1/m1* gene showed a significant association with severe active chronic gastritis[59].

The *iceA* gene has two alleles: *iceA1* and *iceA2*. The expression of *iceA1* is regulated by the contact of *H. pylori* with epithelial cells of the gastric mucosa and is associated with PUD and GC, whereas expression of *iceA2* is related to asymptomatic gastritis[15,53]. We showed in a recent study with strains obtained from patients in Southern Brazil that the *iceA1* allele was related to erosive gastritis, whereas the *iceA2* allele was associated with enanthematous gastritis[39]. Another study in China found that the *iceA1* allele was related to development of GC[60].

The *babA* gene encodes a membrane protein named BabA adhesin that binds to Lewis blood group antigens on the surface of gastric cells[61]. The *babA* gene

is an adhesion factor. One of the characteristics of *H. pylori* that assists in establishing persistent colonization of the gastric epithelium and contributes to its pathogenicity is intimate contact between the bacterium and the epithelium, thereby facilitating the release of pathogenicity factors. Although three alleles of the *bab* gene have been identified (*babA1*, *babA2* and *babB)*, only the *babA2* gene product is able to bind to Lewis b antigen[62]. A recent study showed that the *babA2* gene correlated positively with bacterial density score, inflammation activity and chronic inflammation of the gastric mucosa[63].

## CYTOKINE GENE POLYMORPHISMS AND THEIR INFLUENCE ON GASTRIC DISEASES RELATED TO *Helicobacter pylori* INFECTION

*H. pylori* induces gastric lesions associated with chronic inflammation in the gastric mucosa that are mediated by an array of pro- and anti-inflammatory cytokines[64]. Cytokines are intercellular signaling proteins that serve as key modulators of the immune system and inflammatory process[65]. Polymorphisms in genes that encode pro-inflammatory cytokines such as IL-1β, IL-6 and IL-8 and anti-inflammatory cytokines such as IL-1Ra and IL-10 directly influence inter-individual variation in the magnitude of the cytokine response. This variation clearly contributes to an individual's ultimate clinical outcome following infection with *H. pylori*[66,67].

In general, during *H. pylori* infection gastric inflammation is increased and acid production is potently inhibited in patients with high producer alleles of pro-inflammatory cytokines and low producer alleles of anti-inflammatory cytokines. This combination results in a higher risk for the development of atrophic gastritis, gastric ulcer or GC. In contrast, low producer allele carriers of pro-inflammatory cytokines

and high producer allele carriers of anti-inflammatory cytokines have decreased inflammation and weak acid inhibition, resulting in mild gastric inflammation[68].

The *IL-1* gene family contains the genes *IL-1A, IL-1B* and *IL-1RN* on the long arm of human chromosome 2 distributed over a region of 430-kilobases[69]. *IL-1B* encodes IL-1β, a pro-inflammatory cytokine and a powerful inhibitor of gastric acid secretion that plays an important role in initiating and amplifying the inflammatory response to *H. pylori* infection[70]. There are three single nucleotide polymorphisms (SNPs) in the *IL-1B* gene: a T-C base transition at position -31 and C-T base transitions at positions -511 and +3954 from the transcriptional start site[71]. The presence of the T allele at positions -511 and +3954 and the C allele at position -31 is associated with high levels of IL-1 secretion[33,72]. These polymorphisms are related with hypochlorhydria, chronic atrophic gastritis, gastric ulcer and GC in response to *H. pylori* infection[64,73-76].

*IL-1RN* encodes the IL-1 receptor antagonist (IL-1Ra), an anti-inflammatory cytokine that competitively binds to *IL-1* receptors and thereby modulates the potentially damaging effects of *IL-1*[19,77]. *IL-1RN* play a decisive role in modulating the risk of developing hypochlorhydria, gastric atrophy and GC in the presence of *H. pylori* infection[76]. The *IL-1RN* gene contains an 86-bp variable number of tandem repeat polymorphisms (VNTR) in intron 2, which leads to the presence of 5 different alleles: allele 1 (4 repeats), allele 2 (2 repeats), allele 3 (5 repeats), allele 4 (3 repeats), and allele 5 (6 repeats)[78,79]. The 4-repeat (IL-1RN*1) and 2-repeat (IL-1RN*2) alleles are the most common, whereas the others appear in less than 5% of individuals[80]. Allele 2 (IL-1RN*2) is associated with enhanced IL-1β production[81]. In a meta-analysis study, Peleteiro et al.[82] reported that the IL-1RN*2 genotype seems to consistently increase the risk of gastric precancerous lesions, supporting a role for

this polymorphism in the early stages of gastric carcinogenesis. Studies in different populations revealed association between individual carriers of allele 2 and increased risk of developing chronic gastritis, gastric ulcers and GC[83-86].

IL-6 is a multifunctional cytokine produced by many types of cells, including monocytes, lymphocytes, fibroblasts, endothelial cells, keratinocytes and endometrial cells[87]. IL-6 acts as an inflammatory mediator and endocrine regulator, playing an important role in host defense mechanisms as a messenger between innate and adaptive systems[88]. Moreover, it is involved in the regulation of various cellular functions, such as proliferation, apoptosis, angiogenesis and differentiation[89]. The *IL-6* gene is located on chromosome 7p21, and a single nucleotide change from G to C at position -174 in the promoter region has been described. This SNP may result in inter-individual variation in transcription and expression of *IL-6*, and therefore influence an individual's susceptibility to a diverse range of diseases. Mucosal IL-6 levels are elevated in *H. pylori*-associated gastritis and diminished after eradication of the infection[68,90]. In 2012, Liu et al.[91] showed an increased cancer risk for individuals with the CC genotype compared to those carrying the GG genotype in African populations.

The pro-inflammatory factor IL-8, a member of the CXC (Cys-X-Cys) chemokine superfamily, is recognized as a neutrophil and lymphocyte chemotactic factor and is an inductor of cell proliferation, migration and angiogenesis[68,92]. Gastric mucosal IL-8 levels significantly increase after *H. pylori* infection parallel to the severity of gastritis[93]. IL-8 plays an important role in the *H. pylori* induced gastric mucosal inflammatory process and induces tissue damage by secreting toxic oxygen metabolites and proteolytic enzymes[94]. The *IL-8* gene, located on chromosome 4q13-q21, is 5.2 kb long and possesses a T-A base transition at position -251 in the

proximal promoter region numbering from the transcription start site. The A allele affects *IL-8* gene transcription and tends to be associated with increased IL-8 production by gastric epithelial cells and more severe inflammation[10,95,96]. In recent years, a number of studies examining the IL-8 -251T/A SNP showed varying results. Recent meta-analyses have suggested that the IL-8 -251T/A polymorphism is associated with increased PUD and GC risk among Asians[96,97]. However, a study examining European patients did not find the correlation between the IL-8 -251T/A SNP and GC[98]. Moreover, a Brazilian study found that individuals with the A/A genotype may have protective effects for GC[99]. These contrasting results suggest that the IL-8 -251T/A SNP may be associated differently with gastric diseases depending on the ethnicity and pathogenicity of *H. pylori* genes.

IL-10 is a pleiotropic anti-inflammatory cytokine produced by activated immune cells, especially monocytes/macrophages, B cells and Th2 cells[100,101]. IL-10 is involved in down-regulation of cell-mediated immune responses and cytotoxic inflammatory responses[102]. It is capable of inhibiting the production of pro-inflammatory cytokines such as IFN-$\gamma$, IL-1, IL-2, IL-3, IL-6, IL-8, TNF-$\alpha$, and GM-CSF, and inducing B-cell proliferation and differentiation[101,103]. The *IL-10* gene is located on chromosome 1q31-1q32[104]. A C-T base transition located at position -819 and C-A base transition at position -592 have been identified in the 5´ flanking region of the *IL-10* gene. These SNPs in the gene promoter are related to different serum level of IL-10 in vivo[102,103]. The C allele at positions -819 and -592 has been associated with high IL-10 production, and the T allele at position -819 and the A allele at position -592 have been related to low IL-10 production[105]. Low IL-10 production in patients infected with *H. pylori* results in increased gastric inflammation intensity, hypochlorhydria and increased risk of gastric atrophy and GC[101]. Previous

studies suggested that the *IL-10* gene -819C/T and -592C/A SNPs may be associated with GC[86,106-108].

## RELATIONSHIP OF ENVIRONMENTAL/BEHAVIORAL FACTORS WITH *Helicobacter pylori* INFECTION

Understanding the epidemiology and mode of transmission of *H. pylori* is important to prevent its spread because this bacterium is present in nearly half the world's population[12,109,110]. The transmission of *H. pylori* occurs from person to person by the oral–oral (through saliva, dental plaque, or backflow of gastric contents) or fecal–oral (through water contamination) routes[109,111].

*H. pylori* infection is mainly acquired in childhood and, unless treated, can remain throughout the life of the individual[112]. Childhood infections, such as tonsillitis, infectious diarrhea and diphtheria, are associated with decreased secretion of gastric acid, as well as malnutrition. Regions where childhood infections and malnutrition are common provide the ideal environment for *H. pylori* colonization[113].

There is a strong correlation between poor socioeconomic conditions and *H. pylori* infection. In general, inadequate sanitation practices, low family income, poor hygiene and household crowding may be related to a higher prevalence of this infection[26,114]. A study in Turkey found that lower education levels of mothers, lower family income, poor living conditions, and higher numbers of siblings were correlated with higher *H. pylori* positivity in children[115]. The transmission of *H. pylori* occurs from infected mothers to their offspring and among siblings, notably from younger siblings to older ones[116]. Another study with Brazilian children showed that number of siblings and nursery attendance were positively associated with *H. pylori* infection[117].

A recent study conducted in six Latin-American countries reported that crowding was positively associated with *H. pylori* infection. The same work showed that unemployed participants or those working at home were more likely to be urea breath test-positive. According to the authors, these findings may indicate the occurrence of repeated transmissions of *H. pylori* (same or different strain) between individuals who live in the same household due to a greater opportunity of personal contact, helping to maintain a high *H. pylori* prevalence during adulthood[26].

In addition to the factors described above, other variables, such as smoking, alcohol consumption and diet, may influence infection by *H. pylori*. Smoking can be involved in the transmission of infection due to handling and sharing of cigarettes among smokers[118]. A population-based prospective study with Japanese men suggested that cigarette smoking and *H. pylori* infection are significant risk factors for GC[119]. The strong oxidizing effect of cigarette smoke is able to dramatically induce oxidative DNA damage[10].

In addition to smoking, alcohol consumption can also be an independent risk factor for GC in high-risk populations[25]. It is postulated that alcohol consumption facilitates *H. pylori* infection presumably by damaging the gastric mucosa and/or promoting *H. pylori* adherence to the gastric mucosa[120]. A cross-sectional study conducted among the inhabitants of Lanyu Island in Taiwan showed that alcohol consumption was associated with *H. pylori* infection[121].

Another factor that influences infection by *H. pylori* is a diet that provides noxious agents capable of contributing to *H. pylori* pathogenicity or protective agents that hamper its activity, as related to the appearance of non-cancer diseases. The protective effect of high fruit and vegetable intake is related to the presence of the antioxidants carotenoids and vitamins, which prevent the formation of nitrosamines

and neutralize the action of preformed nitrosamides, thereby reducing tumor formation. In contrast, a high salt concentration in the stomach destroys the mucosal barrier, favors colonization by *H. pylori*, and leads to inflammation and damage-causing gastritis and diffuse erosion[10]. Inadequate dietary habits are associated with intense neutrophilia, higher degrees of inflammation and development of GC[27].

## CONCLUSION

There is evidence that genes related to bacterial pathogenicity such as *cagA, vacA s1/m1/i1, iceA1,* and *babA2;* host genetic polymorphisms such as IL-1RN*2, IL-1β (-31C) (-511T) (+3954T), IL-8 (-251A), and IL-10 (-592A) (-819T); and environmental/behavioral factors play a major role in inducing severe gastric disorders. However, we know little about the interplay of these three factors in protection or pre-disposition for the development of these disorders. If future research focuses on deciphering the deterministic rules of these interactions, we will be able to predict, treat, and ultimately prevent serious gastroduodenal diseases.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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## 3.2 Manuscrito 2 - *Helicobacter pylori* infection and associated factors.

Manuscrito submetido à Revista Biomédica do Instituto Nacional de Salud de Colombia.

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## ABSTRACT

**Introduction:** *Helicobacter pylori* is linked to gastroduodenal pathologies.
**Objective:** To determine the frequency and potential risk factors of the *H. pylori* infection.
**Material and methods:** A cross-sectional study was conducted, including 227 patients, submitted to upper gastrointestinal endoscopy. A questionnaire was applied to the patients, before endoscopy. The biopsy specimens were obtained from the antrum and gastric body for histology and PCR.The chi-square test was used for the categorical data analysis. *P*-values<0.05 were considered statistically significant.
**Results:** 66.5% patients were positive for *H. pylori.* Based on the questionnaires applied to the patients, it was verified that marital status, smoking, alcohol consumption, toilet, education level and monthly family income had no significant association with the presence of *H. pylori* ($p>0.05$). However, we observed a significant association between the number of persons per household and presence of *H. pylori* ($p=0.04$). A statistically significant relation also was found between *H.pylori* and the patient's age ($p=0.04$) and between the histological and endoscopic diagnoses and the *H. pylori* infection ($p\leq0.01$).
**Conclusions:** We found a significant relation between household crowding and presence of *H. pylori*, which seems facilitate the person-to-person transmission *H. pylori* within families. Our results also suggest a cohort phenomenon. The increase in the frequency of *H. pylori* infection according to age may be due the acquisition of bacterium predominantly in childhood, when the sanitary conditions were deficient, and not during adulthood. Once acquired and untreated, the persistent *H. pylori* infection might have led to the development of severe gastroduodenal diseases.

**Keywords:** *Helicobacter pylori*. Epidemiology. Endoscopic Diagnosis. Histological Diagnosis. Polymerase Chain Reaction

## RESUMEN

**Introducción.** *Helicobacter pylori* está relacionado com patologias gastrointestinales.
**Objetivo.** Determinar la frecuencia y los potenciales factores de riesgos de las infecciones causadas por *H. pylori.*
**Materiales y métodos.** Um estúdio transversal fue realizado, incluyendo 227 pacientes sometidos a endoscopia gastrointestinal. Previo a La endoscopía datos epidemiológicos fueron documentados. Las biopsias fueron colectadas del antro y cuerpo gástrico para análisis histológico y PCR. Las variables categóricas fueron evaluadas mediante el test de Chi cuadrado, $p<0,05$ fueron consideradas estadísticamente significativas.
**Resultados.** 66,5% de los pacientes resultaron positivos para *H. pylori*. La encuesta epidemiológica no arrojo resultados significativos asociados com estado civil, fumadores/no fumadores, consumo de alcohol, instaciones sanitárias, nível educacional y ingreso familiar mensual y la presencia de *H. pylori* ($p>0,05$). Sin embargo, se observo relación significativa ($p=0,04$) entre el número de habitantes por hogar y *H. pylori.* También se encontró significativa a asociación entre la presencia da bacteria con la edad de los pacientes ($p=0,04$) y con el diagnóstico histológico y endoscópico ($p\leq0,01$).
**Conclusiones.** La presencia de *H. pylori* se relaciono estadísticamente com el asinamiento familiar, situación que favorce la transmición bacteriana persona-persona. Este estúdio sugiere um fenômeno de cohorte. El incremento de la frecuencia de *H. pylori* com la edad puede ser debido a la adquisición de la bacteria predominantemente em la infancia cuando las condiciones sanitárias eran deficientes. Una vez adquirida la infección y no es tratada, está infección persistente puede llevar al desarrollo de severas enfermedades gastrointestinales.

**Palabras clave:** *Helicobacter pylori*. Epidemiología. Diagnóstico Endoscópico. Diagnóstico Histológico. Reacción em Cadena de La Polimerasa.

## INTRODUCTION

*Helicobacter pylori* infection is one of the most common chronic bacterial infections in humans and is present in more than half of the world's population (1). The prevalence of *H. pylori* differs significantly among countries, lower prevalence rates have been reported in developed countries while higher prevalence rates are found in developing countries (2,3). In Brazil, previous epidemiological studies on *H. pylori* have shown that the prevalence varies from 63% to 96% (4-9).

The *H. pylori* infection prevalence has been reported to vary worldwide by ethnicity, age, geographical area and socioeconomic conditions (3,10). The exact transmission route is still unknown, however the transmission of *H. pylori* appears to occur by the oral–oral or fecal–oral routes (3,11). This way, the infection is associated with a lack of proper sanitation, poor hygiene, low socioeconomic status, household crowding, absence of hygienic drinking water and lack of indoor plumbing. Besides, it is also related to smoking and alcohol consumption (12-15).

*H. pylori* is linked to a variety of gastroduodenal pathologies including chronic gastritis, peptic ulcer, gastric cancer and mucosa-associated lymphoid tissue (MALT)-lymphoma (1,16). In view of the importance of *H*. *pylori* in causing these diseases, the purpose of this study was to determine the frequency and potential risk factors of *H. pylori* infection among patients with dyspeptic symptoms.

# MATERIAL AND METHODS

## Patients

A cross-sectional study, including 227 patients with dyspeptic symptoms submitted to upper gastrointestinal endoscopy, was carried on between May 2011 and May 2012 in the Integrated Center of Gastroenterology of the University Hospital Dr. Miguel Riet Corrêa Jr., in the city of Rio Grande and also in the Division of Digestive Endoscopy of the University Hospital São Francisco de Paula, in the city of Pelotas, Brazil. This study was approved by the Research Ethics Committee of Area Health (FURG - process number 23116.001044/2011-16) and carried out in accordance with the ethical standards outlined in the Helsinki Declaration. Written informed consent was obtained from all patients.

## Endoscopic Procedure

Before endoscopy, a questionnaire was applied to the patients. It included questions regarding age, gender, marital status, number of people living in the house, type of consumed water, sanitary, educational level, monthly family income, smoking status and alcohol consumption.

During endoscopy, eight biopsy specimens were obtained from each patient. Of these, four were destined to histology (two from the gastric antrum and two from the gastric body) whereas the other four were intended for PCR (two from the gastric antrum and two from the gastric body). After collection, the biopsy specimens destined for histology were fixed in formalin 10% and the ones intended for PCR were kept in Brain Heart Infusion Broth with 20% glycerol and stored at -70 °C for further DNA extraction.

The endoscopic diagnosis was established in accordance to the Sydney System Classification (17).

### Histological Examination

The biopsy specimens were stained with Hematoxylin-Eosin (H&E) and Giemsa. The histological diagnosis was established according to the Sydney System Classification (18).

### Detection of *H. pylori* infection

The presence of *H*. *pylori* infection in the patients was determined by histological examination and detection of the *ureA* and *ureC* genes by PCR. Patients were considered *H. pylori*-infected if histology and PCR were positive.

### Extraction of DNA

The DNA was extracted from biopsy specimens using DNAzol® Reagent and 10 μg/μL of Proteinase K. The specimens were separated from the broth and resuspended in 100 μL of Proteinase K and 500 μL of DNAzol® Reagent. The mixture was incubated at 55 °C for 3 h, and subsequently, 500 μL of DNAzol® Reagent was added to it again. After centrifugation at 14,000*g* for 10 min, the supernatant was collected and 500 μL cold absolute ethanol was added, followed by another centrifugation at 12,000*g* for 10 min, after which the supernatant was discarded. The DNA pellet was washed two times with 800 μL of 75% ethanol, air dried and resuspended in 50 μL of 8 mM NaOH. The DNA was stored at -20 °C until used.

### Detection of *ureA* gene

The total DNA of the biopsy specimens of each patient was amplified with the primers UREA1 (5'-GCCAATGGTAAATTAGTT-3') and UREA2 (5'–CTCCTTAATTGTTTTTAC–3'). These primers amplify a fragment of 394 bp of *ureA*. The PCR was performed as described by Rota *et al*. (19).

### Detection of *ureC* gene

The total DNA of the biopsy specimens of each patient was amplified using the primers GLM-MF (5´-GGATAAGCTTTTAGGGGTGTTAGGGG-3´) and GLM-MR (5´-GCATTCACAAACTTATCCCCAATC-3´), that amplify a fragment of 140 bp of *ureC*. The PCR was executed as proposed by Espinoza *et al.* (20).

### Statistical Analysis

The results were presented as mean ± standard deviation for continuous variables and percentage (frequencies) for categorical variables. The chi-square test was used for the categorical data analysis. *P*-values <0.05 were considered statistically significant. The statistical tests were carried out with the software Stata version 10.

## RESULTS

Of the 227 patients included in the present study, 55.1% (125) were female and 44.9% (102) were male.The average age was 53.4 ± 13.9 years old with a range of 20–88 years. *H. pylori* was present in 66.5% (151) of the patients. No significant

difference was detected between gender and *H. pylori* infection (female: 53.0% (80), male: 47.0% (71); $p$=0.37). However, a statistically significant relation was observed between the presence of *H. pylori* infection and the patient's age ($p$=0.04). Figure 1 shows *H. pylori* infection according to different age groups. The frequency of *H. pylori* infection increased with age until the age range of 40–59 years, where the infection was more frequent. After this age grouping, the frequency decreased slightly.

Based on the questionnaires applied to the patients, it was verified that marital status, smoking, alcohol consumption, toilet, education level and monthly family income were not significantly associated with *H. pylori* infection status ($p$>0.05). However, we observed a statistically significant relation between the number of persons per household and presence of *H. pylori* ($p$=0.04) (Table 1).

As show in Table 2, a statistically significant association was found between the histological and endoscopic diagnoses and the *H. pylori* infection ($p$<0.05). According to the histological reports, none of the patients infected with *H. pylori* presented the gastric mucosa without an inflammatory infiltrate, in addition, chronic pangastritis was identified in 77.0% of the patients tested positive for the bacterium. Furthermore, based on the endoscopic reports, enanthematous gastritis and peptic ulcer were the most frequent diagnostic, and appeared in 78.3% and 75.5% of the *H. pylori* positive patients, respectively.

## DISCUSSION

The frequency of *H. pylori* infection varies strongly worldwide[3]. In the present study, *H. pylori* infection was observed in 66.5% of the patients. Such frequency was higher than that observed in developed countries like Australia (22.3%) and Denmark

(20.1%), but is similar to the frequency reported earlier in Brazil (78.0%) and in other developing countries, such as Colombia (63.3%), Mexico (60.1%) and Venezuela (68.3%) (21-26).

Our study did not find any significant association between gender and *H. pylori* infection status ($p$=0.37). However, previous studies showed a male predominance in *H. pylori* infection, because a stronger protective immune response to such bacterium could occur in women (27,28).

Figure 1 shows the *H. pylori* infection behavior according to different age groups. A statistically significant association was observed between the patient's age and the presence of *H. pylori* infection ($p$=0.04). The frequencies of *H. pylori* in the different age groups were: 19.9% (20-39), 54.3% (40-59), 23.8% (60-79), and 2.0% (>80). As observed, the infection frequency increase with age may be due to a birth cohort effect (i.e. a change in the rate of infection in childhood). It has been suggested that *H. pylori* infection is acquired mainly during childhood (29-31). Hence, the living conditions throughout childhood seem to play a significant role in the risk of infection. The decrease in the frequency of infection among more recent generations can be related to an improvement of hygiene and also of the socio-economic condition of the population (24). The decrease in the frequency of *H. pylori* observed from the age group of 60-79 years onwards can be explained by the decreased number of microorganisms as a consequence of the cumulative use of antibiotics or of the gastric mucosa atrophy (5,32). This atrophy leads to a pH increase in the stomach, an event which can create an unfavorable environment for *H. pylori* survival (33).

In the current study, we observed that marital status, smoking, alcohol consumption, toilet, education level and monthly family income did not differ

significantly between the patients infected with *H. pylori* and the ones not infected ($p>0.05$), confirming previous similar studies (30-32). On the other hand, we detected a significant relation between household crowding and presence of *H. pylori* ($p=0.04$). This finding may indicate the occurrence of transmission of *H. pylori* between the individuals who live in the same household, due to a greater opportunity of personal contact, helping to maintain a high *H. pylori* prevalence.

A statistically significant association also was found between the histological and endoscopic diagnoses and the *H. pylori* infection ($p<0.05$). According to the histological reports, no patient infected with *H. pylori* had a gastric mucosa without an inflammatory infiltrate and 77.0% of the patients with chronic pangastritis were positive for the bacterium. Based on the endoscopic reports, 78.3% of the patients with enanthematous gastritis and 75.5% of the patients with peptic ulcer were *H. pylori*-positive. The high frequency of peptic ulcer related with *H. pylori* identified in the present study suggests a low influence of acetylsalicylic acid, non-steroid anti-inflammatories and alcohol in the peptic ulcer development (34,35).

As shown in table 2, 55.6% of the patients with gastric cancer were diagnosed with *H. pylori.* As gastric cancer is a multifactorial disease, environmental factors (mainly diet) and host-related variables (e.g. interleukin polymorphisms) may also have been involved in the development of the gastric cancer detected in the patients of this survey (36-39). However, it is important to note that during the atrophic gastritis, intestinal metaplasia and dysplasia, disorders that precede the development of gastric cancer, there is a marked decrease in the *H. pylori* colonization of the gastric mucosa, what could have led to false negative results in the patients with gastric cancer (6,40,41).

To conclude, this study showed a frequency of *H. pylori* infection of 66.5% in a group of 227 Brazilian patients submitted to upper gastrointestinal endoscopy. We found a significant association between household crowding and presence of *H. pylori* infection, which seems to facilitate person-to-person transmission of *H. pylori* within families. Our results also suggest a cohort phenomenon. The increase in the frequency of *H. pylori* infection according to age may be due to the acquisition of this bacterium predominantly in childhood, when the sanitary conditions were deficient, and not during adulthood. Once acquired and untreated, the persistent *H. pylori* infection might have led to the development of gastritis, peptic ulcer or gastric cancer.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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**Table 1.** Factors for *Helicobacter pylori* infection available in the study.

| Study factors | *H. pylori* positive patients (n = 151) % (Number) | *H. pylori* negative patients (n = 76) % (Number) | *p* - value |
|---|---|---|---|
| Marital status | | | |
| Unmarried | 22.5% (34) | 18.4% (14) | |
| Married | 54.3% (82) | 50.0% (38) | 0.38 |
| Divorced/Widower | 23.2% (35) | 31.6% (24) | |
| Smoking | | | |
| No | 74.8% (113) | 84.2% (64) | |
| Yes | 25.2% (38) | 15.8% (12) | 0.11 |
| Alcohol consumption | | | |
| No | 80.1% (121) | 78.9% (60) | |
| Yes | 19.9% (30) | 21.1% (16) | 0.83 |
| $N^0$ of persons per household | | | |
| 1 – 3 | 59.6% (90) | 73.7% (56) | |
| 4 or more | 40.4% (61) | 26.3% (20) | 0.04 |
| Source of water for drinking | | | |
| Public tap | 72.8% (110) | 61.8% (47) | |
| Bottled | 19.2% (29) | 23.7% (18) | 0.17 |
| Well | 8.0% (12) | 14.5% (11) | |
| Toilet | | | |
| Flush toilet | 96.7% (146) | 100.0% (76) | |
| Pit toilet | 2.0% (3) | 0% (0) | 0.28 |
| No toilet | 1.3% (2) | 0% (0) | |
| Years of school | | | |
| 0-4 | 39.7% (60) | 36.8% (28) | |
| 5-8 | 32.5% (49) | 31.6% (24) | 0.83 |
| 9 or more | 27.8% (42) | 31.6% (24) | |
| Monthly family income (US$) | | | |
| 0 – 500 | 53.6% (81) | 51.3% (39) | |
| 501 – 1000 | 32.5% (49) | 29.0% (22) | 0.51 |
| 1001 or more | 13.9% (21) | 19.7% (15) | |

**Table 2.** Association between endoscopic and histological diagnoses and *Helicobacter pylori* infection.

| | *H. pylori* positive patients (n = 151) | *H. pylori* negative patients (n = 76) | *p* - value |
|---|---|---|---|
| **Histological Diagnosis** | | | |
| Gastric mucosa without an inflammatory infiltrate (n = 20) | 0% (0) | 100.0% (20) | |
| Chronic gastritis (n = 42) | 57.1% (24) | 42.9% (18) | <0.001 |
| Chronic pangastritis (n = 165) | 77.0% (127) | 23.0% (38) | |
| **Endoscopic Diagnosis** | | | |
| Normal gastric mucosa (n = 38) | 60.5% (23) | 39.5% (15) | |
| Enanthematous gastritis (n = 69) | 78.3% (54) | 21.7% (15) | |
| Erosive gastritis (n = 62) | 51.6% (32) | 48.4% (30) | 0.01 |
| Peptic ulcer disease (n = 49) | 75.5% (37) | 24.5% (12) | |
| Gastric carcinoma (n = 9) | 55.6% (5) | 44.4% (4) | |

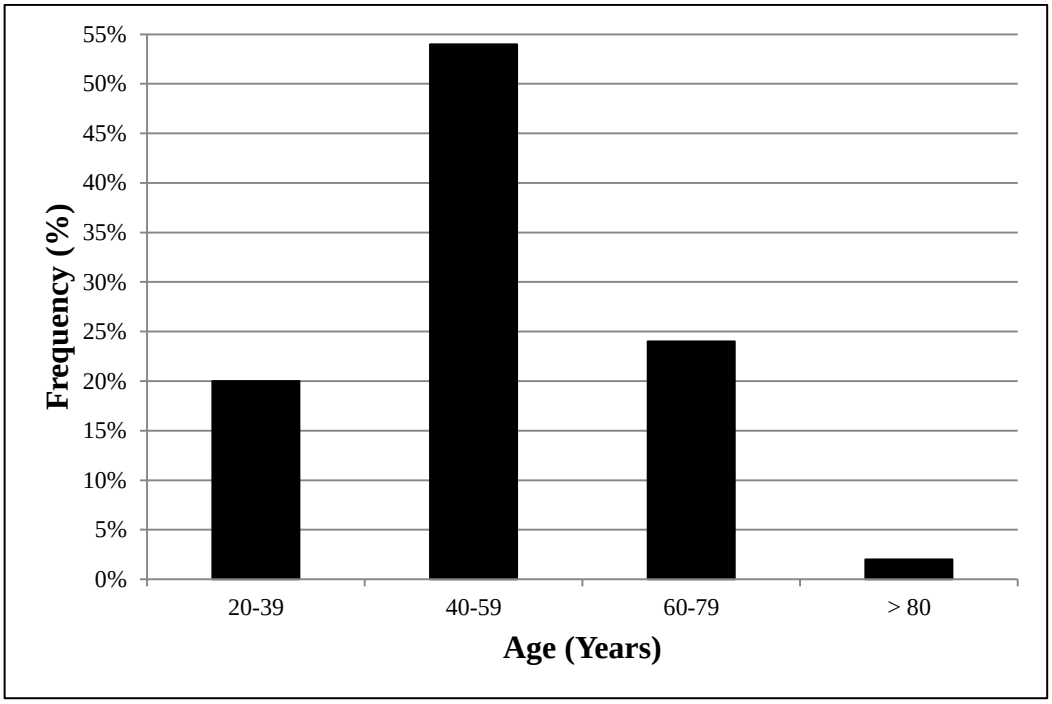


**Figure 1.** Frequency of *Helicobacter pylori* infection according to age.

## 3.3 Manuscrito 3 - Relationship of *interleukin-1B* gene promoter region polymorphism with *Helicobacter pylori* infection and gastritis.

Manuscrito submetido ao Journal of Infection in Developing Countries.

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## ABSTRACT

Introduction: *Helicobacter pylori* infection is associated with gastritis, peptic ulcer disease and gastric carcinoma. The severity of damage is determined by the interplay between environmental/behavioral factors, bacterial pathogenicity genes and host genetic polymorphisms that can influence the secretion levels of inflammatory cytokines. Accordingly, this study aimed to identify polymorphisms in the *IL-1B* and *IL-1RN* genes and their associations with *H. pylori* infection, *cagA* gene of *H. pylori,* and gastroduodenal diseases.
Methodology: We analyzed gastric biopsy samples from 151 patients infected with *H. pylori* and 76 uninfected individuals. *H. pylori* infection was diagnosed by histology and PCR. Polymorphisms at positions -511, -31 and +3954 of the *IL-1B* gene were detected by PCR-RFLP, and an analysis of the VNTR polymorphism of the *IL-1RN* gene was performed by PCR.
Results: We observed that the presence of the T/T genotype at position -511 and the C/C genotype at position -31 were associated with *H. pylori* infection and with an increased risk of gastritis in *H. pylori*-positive patients. Additionally, strains from patients *H. pylori* positive carrying the *cagA* gene was significantly related with the T/T genotype at position -511 of *IL-1B*. We did not find association of polymorphisms at position +3954 of *IL-1B* and in the *IL-1RN* with *H. pylori* infection and with risk of severe gastric diseases.
Conclusions: We demonstrated that polymorphisms in the promoter region of the *IL-1B* gene (at positions -511 and -31) are associated with an enhanced risk of *H. pylori* infection as well as gastritis in *H. pylori*-positive patients.

**Keywords:** *Helicobacter pylori*; Genetic Polymorphism; *Interleukin-1* gene; *cagA* gene; Gastric Diseases.

## Introduction

*Helicobacter pylori* is a bacterium that infects the gastric mucosa of more than half of the world's human population [1]. *H. pylori* infection has been associated with a variety of diseases, such as chronic gastritis, peptic ulcer and gastric carcinoma. However, the majority of people infected with *H. pylori* are asymptomatic, and only 15–20% of them develop such gastroduodenal diseases [2]. It has been hypothesized that a combination of environmental/behavioral variables, host genetic factors and bacterial pathogenicity genes determine the clinical outcome of *H. pylori* infection and the severity of gastric damage [3].

*H. pylori* is genetically a highly diverse microorganism, and one of its major pathogenicity factors is the immunogenic protein CagA (cytotoxin-associated gene A), encoded by the *cagA* gene [4,5]. This gene is located at one end of a 40-kb DNA insertion called *cag* pathogenicity island (*cag*PAI), which encodes the components of the type IV secretion system (T4SS) that forms a pilus for the injection of pathogenicity factors into host target cells, including the CagA oncoprotein [6,7]. Patients infected with *cagA*-positive strains show a greater degree of inflammation of the gastric mucosa, severe atrophic gastritis and a higher risk of gastric carcinoma [8,9].

In addition to bacterial factors, the host immune system also appears to play an important role in the pathogenesis of gastroduodenal diseases by regulating the nature and intensity of the inflammatory response to *H. pylori* infection [10]. The inflammatory cells recruited to the gastric mucosa during infection produce several pro-and anti-inflammatory cytokines, and the secretion levels of these cytokines are influenced by genetic polymorphisms [11,12]. Among these cytokines, it should be

noted that interleukin (IL)-1 plays a central role in the regulation of immune and inflammatory responses. IL-1 consists of two pro-inflammatory cytokines, IL-1α and IL-1β, and a naturally occurring anti-inflammatory agent, IL-1 receptor antagonist (IL-1Ra) [13,14]; the genes encoding IL-1α (*IL-1A*), IL-1β (*IL-1B*), and IL-1Ra (*IL-1RN*) comprise a cluster on human chromosome 2q. The balance between IL-1β and IL-1Ra is the deciding factor of the degree of inflammation in local tissues, which has an important role in many diseases [15].

IL-1β, a powerful inhibitor of gastric acid secretion, is up-regulated in the presence of *H. pylori* and plays a major role in initiating and amplifying the inflammatory response to this infection [3,16]. Three single nucleotide polymorphisms (SNPs) associated with high levels of IL-1β secretion have been reported in the *IL-1B* gene: a T-C base transition at position -31 and C-T base transitions at positions -511 and +3954 from the transcriptional start site [17-19]. Increased production of IL-1β in the gastric mucosa might result in the enhanced suppression of gastric acid secretion, as well as enhanced inflammation, allowing the expansion of *H. pylori* colonization from the gastric antrum to the corpus. This event may lead to the development of hypochlorhydria, severe gastric atrophy, intestinal metaplasia, dysplasia and, ultimately, gastric carcinoma [17,20].

*IL-1RN* competitively binds to IL-1 receptors and counterbalances the potentially injurious pro-inflammatory effects of *IL-1B* [21,22]. *IL-1RN* shows an 86-bp variable number of tandem repeat (VNTR) polymorphism in intron 2, which leads to the presence of 5 different alleles: allele 1 (4 repeats), allele 2 (2 repeats), allele 3 (5 repeats), allele 4 (3 repeats), and allele 5 (6 repeats). The 4-repeat (*IL-1RN**1) and 2-repeat (*IL-1RN**2) alleles are the most common, whereas the other alleles occur at a combined frequency of < 5% [15]. Allele 2 (*IL-1RN**2) is associated with enhanced IL-

1β production [23]. Polymorphisms of the *IL-1RN* gene have been associated with hypochlorhydria and with severe gastroduodenal lesions in the presence of *H. pylori* infection [24,25].

Based on this background, the genotyping of bacterial strains and the detection of host gene polymorphisms could be important in the early identification of individuals at a high risk of developing severe gastric disorders. Therefore, we performed this study to determine the frequency of polymorphisms in the *IL-1B* and *IL-1RN* genes in patients from South Brazil, to evaluate the relationship of these polymorphisms with *H. pylori* infection, and to investigate the association of these polymorphisms with gastritis, peptic ulcer disease and gastric carcinoma. Moreover, we analyzed the relation of these polymorphisms with *cagA* gene of *H. pylori.*

## Methodology

*Subjects and gastric biopsy specimens*

The present study included a total of 227 patients (125 women and 102 men, with an average age of 53.4 years): 151 *H. pylori*-positive (23 with normal gastric mucosa, 86 with gastritis, 37 with peptic ulcer, 5 with gastric cancer) and 76 *H. pylori*-negative (15 with normal gastric mucosa, 45 with gastritis, 12 with peptic ulcer and 4 with gastric cancer) patients. Eight biopsy specimens were obtained from each patient. Of these, four were destined for histology (two from the gastric antrum and two from the gastric body), whereas the other four samples were intended for use in polymerase chain reaction (PCR) (two from the gastric antrum and two from the gastric body). *H. pylori* infection was diagnosed by histological examination and

detection of the *ureA* and *glmM* genes by PCR, as described below. Patients were considered infected with the bacterium when positive results were obtained by at least two of the three methods and uninfected when the results of all diagnostic tests were negative. The diagnosis of gastroduodenal disease was based on endoscopic and histopathological examinations and established in accordance to the Sydney System Classification.

This work was approved by the Research Ethics Committee of Area Health (FURG - process number 23116.001044/2011-16) and carried out in accordance with the ethical standards outlined in the Helsinki Declaration. Written informed consent was obtained from all patients.

*Histological examination, DNA extraction and PCR*

The gastric biopsy specimens destined for histology were fixed in 10% formalin after collection and were then 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*.

The specimens intended for PCR were kept in Brain Heart Infusion Broth with 20% glycerol after collection and stored at -70 °C for further extraction. DNA was extracted using DNAzol® Reagent and 10 µg/µL of Proteinase K, as described previously by Fonseca *et al.* [26]. The integrity of the DNA extracted was assessed by amplification of a 110-bases pairs (bp) fragment specific for human β-globin using initiator oligonucleotides and methodology reported in other study [27]. *H. pylori* infection was diagnosed by PCR using two sets of initiator oligonucleotides: UREA1–UREA2, which amplifies a 394-bp fragment corresponding to the *ureA* gene [28]; and

GLM/MF-GLM/MR, which amplifies a 140-bp fragment corresponding to the *glmM* gene [29].

*Detection of the* cagA *gene*

The *cagA* gene was investigated in the gastric biopsy specimens from *H. pylori* positive patients and was present in the samples from 60 patients. The primers used were cagA1 (5´-ACCCTAGTCGGTAATGGGTTA-3´) and cagA2 (5´-GTAATTGTCTAGTTTCGC-3´) [30]. The PCR was performed as described by Batista *et al*. [31].

*Identification of* IL-1 *gene polymorphisms*

Polymorphisms at positions -511, -31 and +3954 of the *IL-1B* gene were detected by PCR-RFLP (restriction fragment length polymorphism).

To investigate polymorphism at position -511, we used the initiator oligonucleotides 5´-CTGCATACCGTATGTTCTCTGCC-3´ (forward) and 5´-GGAATCTTCCCACTTACAGATGG-3´ (reverse) [32]. PCR was performed under the conditions described by Erzin *et al*., with minor modifications [32]. The PCR products were digested with the restriction enzyme *Dde*I and then analyzed by electrophoresis on a 2.5% agarose gel with ethidium bromide staining. The genotypes were designated as follows: C/C - two bands of 140 and 49 bp; C/T - four bands of 140, 109, 49 and 31 bp and T/T - three bands of 109, 49 and 31 bp [32].

The initiator oligonucleotides used to analyze polymorphism at position -31 were 5´-AGAAGCTTCCACCAATACTC-3´ (forward) and 5´-AGCACCTAGTTGTAAGGAAG-3´ (reverse) [33]. The PCR was made as previously reported [34]. The PCR products were digested with the restriction enzyme *Alu*I and

then visualized by electrophoresis on a 2% agarose gel with ethidium bromide staining. The genotypes were coded as follows: T/T - two bands of 137 and 102 bp; T/C - three bands of 239, 137 and 102 bp and C/C - a single band of 239 bp [33].

To determine polymorphism at position +3954, the initiator oligonucleotides 5´-GTTGTCATCAGACTTTGACC-3´ (forward) and 5´-TTCAGTTCATATGGACCAGA-3´ (reverse) were used [33]. The PCR was performed under the conditions described by Chiurillo *et al*. [34]. The PCR products were digested with the restriction enzyme *Taq*I and then analyzed by electrophoresis on a 2.5% agarose gel with ethidium bromide staining. The genotypes were designated as follows: C/C - two bands of 135 and 114 bp; C/T - three bands of 249, 135 and 114 bp and T/T - a single band of 249 bp [33].

The analysis of the variable numbers of tandem repeat polymorphism in intron 2 of the *IL-1RN* gene was performed by PCR, followed by electrophoresis on a 2% agarose gel. The initiator oligonucleotides used were 5´-CTCAGCAACACTCCTAT-3´ (forward) and 5´-TCCTGGTCTGCAGGTAA-3´ (reverse) [35]; and the PCR was made as previously reported [5]. Polymorphism was based on the number of repeats of an 86-bp sequence. The alleles were coded conventionally, as follows: allele 1 = four repeats; allele 2 = two repeats; allele 3 = five repeats; allele 4 = three repeats; and allele 5 = six repeats [35]. For statistical analysis purpose and because of the low frequency of alleles 3, 4 and 5, the alleles were categorized into IL-1*RN**2, which contains at least one allele 2 and non-IL-1*RN**2, which does not contain allele 2 [36].

To verify the results obtained by PCR-RFLP for the *IL-1B* gene, 10% of the samples evaluated for each polymorphism were randomly selected and confirmed by sequencing.

*Statistical Analysis*

Data typing was carried out on a bank in the Microsoft Excel 2010 program. A consistency analysis, based on the creation and categorization of variables and verification of frequencies, was performed using SPSS version 18.0. To comparison between proportions, the Chi-Squared Test or Fisher's Exact Test was used. *P*-values less than 0.05 were considered to be statistically significant. The prevalence ratio (PR) with 95% confidence interval (CI) was calculated to evaluate the relationship of cytokine gene polymorphisms with gastroduodenal diseases and with *H. pylori* infection.

## Results

Genetic polymorphisms in *IL-1B* and *IL-1RN* were investigated in all 227 patients. For polymorphism at position -511 of the *IL-1B* gene, 41.0% (93/227) of the subjects showed C/C homozygosity, 48.9% (111/227) showed C/T heterozygosity, and 10.1% (23/227) showed T/T homozygosity. With regard to polymorphism at position -31 of *IL-1B*, 39.2% (89/227) of the patients carried the T/T genotype, 47.6% (108/227) the T/C genotype, and 13.2% (30/227) the C/C genotype. Regarding polymorphism at position +3954 of *IL-1B*, C/C homozygosity was found in 63.5% (144/227) of the subjects, C/T heterozygosity in 33.0% (75/227), and T/T homozygosity in 3.5% (8/227). Polymorphism in the *IL-1RN* gene revealed that 53.3% (121/227) of the patients did not contain allele 2 (non-IL-1*RN**2), whereas 46.7% (106/227) contained at least one allele 2 (IL-1*RN**2).

Table 1 shows the frequencies of *IL-1* gene polymorphisms in the presence and absence of *H. pylori* infection. The presence of the T allele (PR = 1.16; $_{95\%}$CI =

1.02-1.32) and the T/T genotype (PR = 1.40; $_{95\%}$CI = 1.09-1.80) at position -511 of the *IL-1B* gene was associated with *H. pylori* infection, as did the presence of the C allele (PR = 1.20; $_{95\%}$CI = 1.06-1.37) and the C/C genotype (PR = 1.48; $_{95\%}$CI = 1.19-1.86) at position -31 of *IL-1B.* With respect to the polymorphism at position +3954 of *IL-1B* and in *IL-1RN*, we did not observe statistically significant relationship of these polymorphisms with *H. pylori* infection.

The distributions of *IL-1* gene polymorphisms in *H. pylori* positive and negative patients with normal gastric mucosa, gastritis, peptic ulcer disease and gastric carcinoma are presented in Table 2. *H. pylori* positive patients and carriers of the T/T genotype at position -511 and the C/C genotype at position -31 showed an increased risk of gastritis (PR = 1.52 and $_{95\%}$CI = 1.11-2.08, PR = 1.60 and $_{95\%}$CI = 1.21-2.13; respectively). No other association was found between *IL-1* polymorphisms and the risk of gastric disease. Despite this, when we analyzed the patients with peptic ulcer disease, the distribution of the T/T genotype at position -511 was higher in the *H. pylori* positive patients (21.6%) compared with the *H. pylori* negative patients (8.4%), as was the distribution of the C/C genotype at position -31 (29.7% *versus* 8.4%) and the distribution of carriers of the *IL-1RN**2 allele (59.5% *versus* 50.0%). Additionally, when we investigated the patients with gastric carcinoma, the frequencies of the T allele at position -511 of *IL-1B*, the C allele at position -31 of *IL-1B* and *IL-1RN**2 allele carriers were higher in patients infected with *H. pylori* compared with non-infected subjects.

The relationship between the *cagA* gene of *H. pylori* and polymorphisms in *IL-1* was also evaluated (Table 3). Strains from patients *H. pylori* positive carrying the *cagA* gene was significantly associated with the T/T genotype at position -511 of *IL-1B* ($p$=0.046). No other genotype of *IL-1* was related with the *cagA* gene.

## Discussion

The frequencies of polymorphisms in the *IL-1B* and *IL-1RN* genes found in this study were similar to those described in European populations [37,38]. Genetic polymorphisms involved in the regulation of gastric acid secretion may to be associated with host susceptibility to the acquisition of *H. pylori* infection and to the development of gastric pathologies [18,36].

In this study, we observed that polymorphisms in the coding region of *IL-1B* (at position +3954) and in *IL-1RN* were not related to *H. pylori* infection. These finding are in agreement with those reported previously [10,39]. However, we did find that the presence of the T allele and the T/T genotype at position -511 as well as the C allele and the C/C genotype at position -31 were associated with *H. pylori* infection.

The presence of the T allele at position -511 and C allele at position -31 is associated with high levels of IL-1β [18]. IL-1 is a powerful inhibitor of gastric acid secretion; on a molar basis, IL-1 is estimated to be 100-fold more potent than proton pump inhibitors and 6000-fold more potent than $H_2$ receptor antagonists [16,40,41]. The decreased of the acid secretion causes hypochlorhydria, which favors *H. pylori* infection because the secretion of acid is an important host defense mechanism against bacterial colonization in the stomach. In addition, a reduction in the flow of gastric secretion may heighten mucosal damage by allowing the accumulation of bacterial toxins and the products of inflammation that would normally be diluted and flushed out [12,36,42].

The present work showed that *H. pylori* positive patients and carriers of genotypes T/T at position -511 and C/C at position -31 have an increased risk of gastritis. The presence of these genotypes appears to increase the levels of IL-1β

and consequently inhibit gastric acid secretion and amplify the inflammatory response to *H. pylori* infection, which results in severe inflammation. This inflammation may lead to continuous injury to the tissue structure and function and to the development of gastroduodenal diseases of higher severity [16,18,43,44].

Interestingly, we did not observe significant association between IL-1 polymorphisms and increased risk of peptic ulcer disease and gastric carcinoma in the presence of *H. pylori* infection, possibly because of a limitation of this study, which was a low number of patients diagnosed with these disorders. Despite this fact, when we analyzed patients with peptic ulcer disease and gastric carcinoma, the frequency of the T/T genotype at position -511 of *IL-1B* was higher in *H. pylori* positive patients compared with *H. pylori* negative patients, as was the frequency of the C/C genotype at position -31 of *IL-1B* and the frequency of carriers of the 2 allele of *IL-1RN*. Previous studies have already demonstrated a positive relationship between *IL-1B* and *IL-1RN* gene polymorphisms and the development of peptic ulcer disease and gastric carcinoma in *H. pylori* positive patients [22,45,46].

In this research, we also analyzed the relationship between *cagA* gene and *IL-1* polymorphisms because *cagA* positive strains induce higher levels of IL-1 expression than do *cagA* negative strains [47]. We found that strains from *H. pylori* positive patients that carried the *cagA* gene were significantly associated with the T/T genotype at position -511, suggesting a synergic interaction between the bacterium and host polymorphism, with more severe damage of the gastric mucosa [48].

## Conclusions

We demonstrated that polymorphisms in the promoter region of the *IL-1B* gene (at positions -511 and -31) are significantly associated with *H. pylori* infection. Additionally, *H. pylori* positive patients and carriers of the T/T genotype at position -511 and the C/C genotype at position -31 have an increased risk of gastritis but not of peptic ulcer disease and gastric carcinoma, possibly because of the low number of patients diagnosed with ulcer and carcinoma, a limitation of this study. Nonetheless, it is worth noting that in the disorders that precede the development of gastric carcinoma, there is a marked decrease in the colonization of the gastric mucosa by *H. pylori*, which may lead to false negative results in infected patients who develop gastric carcinoma.

More extensive studies investigating polymorphisms in other cytokines with individuals from different ethnicities are required to understand the complex interplay between host and microorganism in the development of gastric disease. The identification of host biomarkers related to protection against or induction of gastric disorder will be able to allow a better prognosis for *H. pylori* infection.

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## Authors' contributions

The authors contributed equally to this study.

## Conflict of interests

No conflict of interests is declared.

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**Table 1.** Frequencies of the polymorphisms of *IL-1* gene in patients *H. pylori* positive and negative.

| | ***H. pylori* positive patients N (%)** | ***H. pylori* negative patients N (%)** | **PR ($_{95\%}$CI)** |
|---|---|---|---|
| **Alleles ➡ *IL-1B* (-511)** | | | ***p* = 0.017**[a] |
| C | 187 (61.9%) | 110 (72.4%) | 1.0 |
| T | 115 (38.1%) | 42 (27.6%) | 1.16 (1.02-1.32) |
| **Genotypes ➡ *IL-1B* (-511)** | | | ***p* = 0.069**[b] |
| C/C | 55 (36.4%) | 38 (50.0%) | 1.0 |
| C/T | 77 (51.0%) | 34 (44.7%) | 1.17 (0.95-1.45) |
| T/T | 19 (12.6%) | 4 (5.3%) | 1.40 (1.09-1.80) |
| **Alleles ➡ *IL-1B* (-31)** | | | ***p* = 0.004**[a] |
| T | 177 (58.6%) | 109 (71.7%) | 1.0 |
| C | 125 (41.4%) | 43 (28.3%) | 1.20 (1.06-1.37) |
| **Genotypes ➡ *IL-1B* (-31)** | | | ***p* = 0.017**[b] |
| T/T | 52 (34.5%) | 37 (48.7%) | 1.0 |
| T/C | 73 (48.3%) | 35 (46.0%) | 1.16 (0.93-1.44) |
| C/C | 26 (17.2%) | 4 (5.3%) | 1.48 (1.19-1.86) |
| **Alleles ➡ *IL-1B* (+3954)** | | | ***p* = 0.106** [a] |
| C | 247 (81.8%) | 116 (76.3%) | 1.0 |
| T | 55 (18.2%) | 36 (23.7%) | 0.89 (0.74-1.06) |
| **Genotypes ➡ *IL-1B* (+3954)** | | | ***p* = 0.311**[b] |
| C/C | 101 (66.9%) | 43 (56.6%) | 1.0 |
| C/T | 45 (29.8%) | 30 (39.5%) | 0.86 (0.69-1.06) |
| T/T | 5 (3.3%) | 3 (3.9%) | 0.89 (0.52-1.54) |
| ***IL-1RN*** | | | ***p* = 0.498**[a] |
| non-*IL-1RN**2 | 81(53.6%) | 40 (52.6%) | 1.0 |
| *IL-1RN*2* | 70 (46.4%) | 36 (47.4%) | 0.99 (0.82-1.19) |

PR: Prevalence ratio; $_{95\%}$CI: 95% confidence interval

[a] *P*-values were determined by Fisher´s Exact Test

[b] *P*-values were determined by Chi-Squared Test

**Table 2.** Frequencies of the polymorphisms of *IL-1* gene in patients *H. pylori* positive and negative with normal gastric mucosa and with different gastric disorders.

| | **Normal Gastric Mucosa** | | | **Gastritis** | | | **Peptic Ulcer Disease** | | | **Gastric Carcinoma** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *H. pylori* positive patients N (%) | *H. pylori* negative patients N (%) | **PR ($_{95\%}$CI)** | *H. pylori* positive patients N (%) | *H. pylori* negative patients N (%) | **PR ($_{95\%}$CI)** | *H. pylori* positive patients N (%) | *H. pylori* negative patients N (%) | **PR ($_{95\%}$CI)** | *H. pylori* positive patients N (%) | *H. pylori* negative patients N (%) | **PR ($_{95\%}$CI)** |
| **Alleles *IL-1B* (-511)** | | | ***p* = 0.747**[b] | | | ***p* = 0.083**[b] | | | ***p* = 0.468**[b] | | | ***p* = 0.195**[b] |
| C | 29 (63.0%) | 20 (66.7%) | 1.0 | 112 (65.1%) | 68 (75.6%) | 1.0 | 40 (54.1%) | 15 (62.5%) | 1.0 | 6 (60.0%) | 7 (87.5%) | 1.0 |
| T | 17 (37.0%) | 10 (33.3%) | 1.06 (0.73-1.54) | 60 (34.9%) | 22 (24.4%) | 1.18 (0.99-1.40) | 34 (45.9%) | 9 (37.5%) | 1.09 (0.87-1.36) | 4 (40.0%) | 1 (12.5%) | 1.73 (0.83-3.61) |
| **Genotypes *IL-1B* (-511)** | | | ***p* = 0.605**[b] | | | ***p* = 0.157**[b] | | | ***p* = 0.584**[b] | | | ***p* = 0.500**[b] |
| C/C | 8 (34.8%) | 7 (46.7%) | 1.0 | 34 (39.5%) | 24 (53.3%) | 1.0 | 11 (29.7%) | 4 (33.3%) | 1.0 | 2 (40.0%) | 3 (75.0%) | 1.0 |
| C/T | 13 (56.5%) | 6 (40.0%) | 1.28 (0.73-2.25) | 44 (51.2%) | 20 (44.5%) | 1.17 (0.89-1.54) | 18 (48.7%) | 7 (58.3%) | 0.98 (0.66-1.45) | 2 (40.0%) | 1 (25.0%) | 1.67 (0.44-6.36) |
| T/T | 2 (8.7%) | 2 (13.3%) | 0.94 (0.32-2.78) | 8 (9.3%) | 1 (2.2%) | 1.52 (1.11-2.08) | 8 (21.6%) | 1 (8.4%) | 1.21 (0.83-1.78) | 1 (20.0%) | - | - |
| **Alleles *IL-1B* (-31)** | | | ***p* = 0.828**[b] | | | ***p* = 0.029**[b] | | | ***p* = 0.286**[b] | | | ***p* = 0.119**[a] |
| T | 28 (60.9%) | 19 (63.3%) | 1.0 | 107 (62.2%) | 68 (75.6%) | 1.0 | 37 (50.0%) | 15 (62.5%) | 1.0 | 5 (50.0%) | 7 (87.5%) | 1.0 |
| C | 18 (39.1%) | 11 (36.7%) | 1.04 (0.72-1.51) | 65 (37.8%) | 22 (24.4%) | 1.22 (1.03-1.45) | 37 (50.0%) | 9 (37.5%) | 1.13 (0.90-1.41) | 5 (50.0%) | 1 (12.5%) | 2.0 (0.94-4.27) |
| **Genotypes *IL-1B* (-31)** | | | ***p* = 0.940**[b] | | | ***p* = 0.061**[b] | | | ***p* = 0.306**[b] | | | ***p* = 0.242**[b] |
| T/T | 8 (34.8%) | 6 (40.0%) | 1.0 | 32 (37.2%) | 24 (53.3%) | 1.0 | 11 (29.7%) | 4 (33.3%) | 1.0 | 1 (20.0%) | 3 (75.0%) | 1.0 |
| T/C | 12 (52.2%) | 7 (46.7%) | 1.11 (0.63-1.95) | 43 (50.0%) | 20 (44.5%) | 1.19 (0.90-1.58) | 15 (40.6%) | 7 (58.3%) | 0.93 (0.61-1.41) | 3 (60.0%) | 1 (25.0%) | 3.0 (0.50-17.95) |
| C/C | 3 (13.0%) | 2 (13.3%) | 1.05 (0.45-2.45) | 11 (12.8%) | 1 (2.2%) | 1.60 (1.21-2.13) | 11 (29.7%) | 1 (8.4%) | 1.25 (0.88-1.73) | 1 (20.0%) | - | - |
| **Alleles *IL-1B* (+3954)** | | | ***p* = 0.467**[b] | | | ***p* = 0.365**[b] | | | ***p* = 0.491**[b] | | | ***p* = 0.617**[a] |
| C | 37 (80.4%) | 22 (73.3%) | 1.0 | 140 (81.4%) | 69 (76.7%) | 1.0 | 63 (85.1%) | 19 (79.2%) | 1.0 | 7 (70.0%) | 6 (75.0%) | 1.0 |
| T | 9 (19.6%) | 8 (26.7%) | 0.84 (0.52-1.38) | 32 (18.6%) | 21 (23.3%) | 0.90 (0.71-1.14) | 11 (14.9%) | 5 (20.8%) | 0.89 (0.63-1.27) | 3 (30.0%) | 2 (25.0%) | 1.11 (0.46-2.67) |
| **Genotypes *IL-1B* (+3954)** | | | ***p* = 0.761**[b] | | | ***p* = 0.380**[b] | | | ***p* = 0.334**[a] | | | ***p* = 0.785**[a] |
| C/C | 15 (65.2%) | 8 (53.3%) | 1.0 | 57 (66.3%) | 25 (55.6%) | 1.0 | 26 (70.3%) | 7 (58.3%) | 1.0 | 3 (60.0%) | 3 (75.0%) | 1.0 |
| C/T | 7 (30.4%) | 6 (40.0%) | 0.83 (0.46-1.48) | 26 (30.2%) | 19 (42.2%) | 0.83 (0.62-1.11) | 11 (29.7%) | 5 (41.7%) | 0.87 (0.60-1.27) | 1 (20.0%) | - | - |
| T/T | 1 (4.4%) | 1 (6.7%) | 0.77 (0.18-3.16) | 3 (3.5%) | 1 (2.2%) | 1.08 (0.60-1.93) | - | - | - | 1 (20.0%) | 1 (25.0%) | 1 (0.20-4.95) |
| ***IL-1RN*** | | | ***p* = 0.376**[b] | | | ***p* = 0.668**[b] | | | ***p* = 0.565**[b] | | | ***p* = 0.357**[a] |
| non-*IL-1RN*2* | 11 (47.8%) | 5 (33.3%) | 1.0 | 53 (61.6%) | 26 (57.8%) | 1.0 | 15 (40.5%) | 6 (50.0%) | 1.0 | 2 (40.0%) | 3 (75.0%) | 1.0 |
| *IL-1RN*2* | 12 (52.2%) | 10 (66.7%) | 0.79 (0.48-1.31) | 33 (38.4%) | 19 (42.2%) | 0.95 (0.73-1.22) | 22 (59.5%) | 6 (50.0%) | 1.10 (0.79-1.53) | 3 (60.0%) | 1 (25.0%) | 1.88 (0.56-6.31) |

PR: Prevalence ratio; $_{95\%}$CI: 95% confidence interval

[a] *P*-values were determined by Fisher´s Exact Test

[b] *P*-values were determined by Chi-Squared Test

**Table 3.** Frequencies of the polymorphisms of *IL-1* gene in *cagA* positive and *cagA* negative patients.

| | ***cagA* positive patients N (%)** | ***cagA* negative patients N (%)** | **PR ($_{95\%}$CI)** |
|---|---|---|---|
| **Alleles ➡ *IL-1B* (-511)** | | | ***p* = 0.248**[a] |
| C | 71 (59.2%) | 116 (63.7%) | 1.0 |
| T | 49 (40.8%) | 66 (36.3%) | 1.12 (0.85-1.49) |
| **Genotypes ➡ *IL-1B* (-511)** | | | ***p* = 0.046**[b] |
| C/C | 23 (38.3%) | 32 (35.2%) | 1.0 |
| C/T | 25 (41.7%) | 52 (57.1%) | 0.78 (0.50-1.22) |
| T/T | 12 (20.0%) | 7 (7.7%) | 1.51 (0.95-2.40) |
| **Alleles ➡ *IL-1B* (-31)** | | | ***p* = 0.249**[a] |
| T | 67 (55.8%) | 110 (60.4%) | 1.0 |
| C | 53 (44.2%) | 72 (39.6%) | 1.12 (0.85-1.48) |
| **Genotypes ➡ *IL-1B* (-31)** | | | ***p* = 0.213**[b] |
| T/T | 21 (35.0%) | 31 (34.1%) | 1.0 |
| T/C | 25 (41.7%) | 48 (52.7%) | 0.85 (0.54-1.34) |
| C/C | 14 (23.3%) | 12 (13.2%) | 1.33 (0.82-2.17) |
| **Alleles ➡ *IL-1B* (+3954)** | | | ***p* = 0.342**[a] |
| C | 100 (83.3%) | 147 (80.8%) | 1.0 |
| T | 20 (16.7%) | 35 (19.2%) | 0.90 (0.61-1.31) |
| **Genotypes ➡ *IL-1B* (+3954)** | | | ***p* = 0.655**[b] |
| C/C | 41 (68.3%) | 60 (65.9%) | 1.0 |
| C/T | 18 (30.0%) | 27 (29.7%) | 0.99 (0.64-1.51) |
| T/T | 1 (1.7%) | 4 (4.4%) | 0.49 (0.08-2.89) |
| ***IL-1RN*** | | | ***p* = 0.075**[a] |
| non-*IL-1RN**2 | 37 (61.7%) | 44 (48.4%) | 1.0 |
| *IL-1RN**2 | 23 (38.3%) | 47 (51.6%) | 0.72 (0.48-1.08) |

PR: Prevalence ratio; $_{95\%}$CI: 95% confidence interval

[a] *P*-values were determined by Fisher´s Exact Test

[b] *P*-values were determined by Chi-Squared Test

## 3.4 Manuscrito 4 - Gene polymorphism of *IL-6, IL-8* and *IL-10* and the risk of gastric pathologies in patients infected with *Helicobacter pylori*.

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## ABSTRACT

*Background/Purpose: H. pylori*-induced gastric mucosal inflammation is mediated by pro- and anti-inflammatory cytokines. Polymorphisms in genes that code for cytokines influence secretion levels and appear to contribute to the risk of gastric diseases. Accordingly, we performed this study to identify 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 from 151 patients infected with *H. pylori* and 76 uninfected patients were used. *H. pylori* infection was diagnosed by histological examination and detection of the *ureA* and *glmM* genes. Polymorphisms in *IL-6* (at position -174)*, IL-8* (at position -251) and *IL-10* (at positions -819 and -592) were detected by PCR-RFLP.
*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. Additionally, only carriers of the A/A genotype at position -251 of *IL-8*, the T allele at position -819 of *IL-10* and the A allele at position -592 of *IL-10* showed increased risk of peptic ulcer disease in the presence of *H. pylori* infection. We did not find a relationship between polymorphisms in the *IL-6, IL-8* and *IL-10* genes and a higher risk of gastric carcinoma.
*Conclusion:* We demonstrated that polymorphisms in the *IL-8* gene are significantly associated with *H. pylori* infection. Moreover, polymorphisms in the *IL-8* and *IL-10* genes are related to an enhanced risk of peptic ulcer disease in *H. pylori* positive patients.

**Keywords:** Genetic Polymorphism; *Helicobacter pylori*; *Interleukin-6* gene; *Interleukin-8* gene; *Interleukin-10* gene

## Introduction

*Helicobacter pylori* is a bacterium that causes various diseases, such as chronic gastritis, peptic ulcer disease, gastric carcinoma and mucosa-associated lymphoid tissue (MALT)-lymphoma.[1] 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.[2,3] Polymorphisms in genes that encode cytokines such as interleukin (IL)-6, IL-8 and IL-10 influence secretion levels and appear to contribute to the risk of developing gastroduodenal disease.[4-6]

IL-6 is a pro-inflammatory cytokine that functions as an inflammatory mediator and endocrine regulator. In addition, IL-6 plays an important role in host defense mechanisms as a messenger between innate and adaptive systems.[7] The *IL-6* gene is located on chromosome 7, and a polymorphism in the 5´ flanking region, at position -174 (G→C), has been described. This polymorphism may result in inter-individual variation in the transcription and expression of *IL-6*, and increased gastric mucosal levels of IL-6 have been reported in *H. pylori* positive individuals with gastritis and gastric carcinoma.[3,8-10]

Another pro-inflammatory cytokine that has an important role in the pathogenesis of *H. pylori*-induced diseases is IL-8, a potent chemoattractant for neutrophils.[11] The *IL-8* gene, located on chromosome 4, exhibits a single nucleotide polymorphism (SNP → T-A base transition) at -251 nucleotide relative to the transcriptional start site.[12,13] The A allele tends to be associated with increased IL-8 production and consequently with an amplified inflammatory response.[3,12]

Unlike of the two cytokines mentioned above, IL-10 is an anti-inflammatory cytokine that downregulates cell-mediated immune responses and cytotoxic inflammatory responses.[14] The gene encoding human IL-10 is located on chromosome 1. Two SNPs 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.[15,16] Low IL-10 production is associated with increased gastric inflammation intensity and an enhanced risk of peptic ulcer disease and gastric carcinoma[17,18].

In the present study, we aimed to determine the frequency of polymorphisms in the *IL-6, IL-8* and *IL-10* genes, to analyze the association of these polymorphisms with *H. pylori* infection and to investigate the relationship between these polymorphisms and the risk of gastritis, peptic ulcer disease and gastric carcinoma.

## Methods

### Patients and Gastric Biopsy Samples

The present study included a total of 227 patients (125 women and 102 men with an average age of 53.4 years): 151 *H. pylori* positive (23 with normal gastric mucosa, 86 with gastritis, 37 with peptic ulcer, 5 with gastric cancer) and 76 *H. pylori* negative (15 with normal gastric mucosa, 45 with gastritis, 12 with peptic ulcer and 4 with gastric cancer) individuals. Eight biopsy samples were obtained from each patient. Of these, four were destined for histology (two from the gastric antrum and two from the gastric body), whereas the other four were intended for use in polymerase chain reaction (PCR) (two from the gastric antrum and two from the gastric body). *H. pylori* infection was diagnosed by histology and detection of the

*ureA* and *glmM* genes by PCR, as described below. Patients were considered infected with the bacterium when positive results were obtained by at least two of the three methods and 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 to the Sydney System Classification.

This work was approved by the Research Ethics Committee of Area Health (FURG - process number 23116.001044/2011-16), and written informed consent was obtained from all patients.

**Histology, DNA Extraction and PCR**

The gastric biopsy samples analyzed by histology were fixed in formalin 10% after collection and 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*.

The samples intended for PCR were kept after collection in Brain Heart Infusion Broth with 20% glycerol and stored at -70 °C for further extraction. DNA was extracted using DNAzol® Reagent and 10 μg/μL of Proteinase K, as described previously by Fonseca et al.[19] The integrity of extracted DNA was assessed by amplification of a 110 bases pairs (bp) fragment of human β-globin using of primers and methodology already reported in another study.[20] *H. pylori* infection was diagnosed by PCR using two sets of primers: UREA1–UREA2, which amplifies a 394 bp fragment corresponding to the *ureA* gene;[21] and GLM/MF-GLM/MR, which amplifies a 140 bp fragment corresponding to the *glmM* gene.[22]

**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 polymorphism at position -174 of the *IL-6* gene, we used the primers 5´-TTGTCAAGACATGCCAAAGTG-3´ (forward) and 5´-TCAGACATCTCCAGTCCTATA-3´ (reverse).[23] PCR was performed under the conditions described by Gatti et al.[23] The PCR products were digested with the restriction enzyme *Nla*III and then analyzed by electrophoresis on a 3.5% agarose gel stained with ethidium bromide. The genotypes were designated as follows: G/G - three bands of 233, 54 and 13 bp; G/C - five bands of 233, 122, 111, 54 and 13 bp and C/C - four bands of 122, 111, 54 and 13 bp.[23]

To analyze polymorphism at position -251 of the *IL-8* gene, the primers 5´-TTCTAACACCTGCCACTCTAG-3´ (forward) and 5´-CTGAAGCTCCACAATTTGGTG-3´ (reverse) were used; the PCR conditions utilized were as reported in another study.[24] The PCR products were digested with the restriction enzyme *Mfe*I and then visualized by electrophoresis on a 3.5% agarose gel stained with ethidium bromide. The genotypes were coded as follows: T/T - a single band of 108 bp; T/A - three bands of 108, 76 and 32 bp and A/A - two bands of 76 and 32 bp.[24]

To determine polymorphism at position -819 of the *IL-10* gene, we used the primers 5´-ATCCAAGACAACACTACTAA-3´ (forward) and 5´-TAAATATCCTCAAAGTTCC-3´ (reverse) and PCR was performed under the conditions described by Cheng et al.[25] The PCR products were digested with the restriction enzyme *Mae*III and then analyzed by electrophoresis on a 2.5% agarose gel stained with ethidium bromide. The genotypes were designated as follows: C/C -

three bands of 292, 217 and 79 bp; C/T - four bands of 509, 292, 217 and 79 bp and T/T - two bands of 509 and 79 bp.[25]

To identify polymorphism at position -592 of the *IL-10* gene, primers 5´-GACTCCAGCCACAGAAGCTTA-3´ (forward) and 5´-ATATCCTCAAAGTTCCCAAGC-3´ (reverse) were utilized, and the PCR conditions used were as previously reported.[25] The PCR products were digested with the restriction enzyme *Rsa*I and then visualized by electrophoresis on a 2.5% agarose gel stained with ethidium bromide. The genotypes were coded as follows: C/C - a single band of 302 bp; C/A - three bands of 302, 240 and 62 bp and A/A - two bands of 240 and 62 bp.[25]

To verify the results obtained by PCR-RFLP for the *IL-6, IL-8* and *IL-10* genes, 10% of the samples evaluated for each polymorphism were randomly selected and confirmed by sequencing.

**Statistical Analysis**

Data typing was carried out in the Microsoft Excel 2010 program. A consistency analysis, based on the creation and categorization of variables and verification of frequencies, was performed using SPSS version 18.0. To comparison between proportions, the Chi-Squared Test or Fisher's Exact Test was used. *P*-values less than 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 and gastroduodenal disease and *H. pylori* infection.

## Results

Genetic polymorphisms in *IL-6, IL-8* and *IL-10* were investigated in all study subjects. For polymorphism at position -174 of the *IL-6* gene, 54.6% (124/227) of the patients carried the G/G genotype, 37.0% (84/227) the G/C genotype and 8.4% (19/227) the C/C genotype. With regard to polymorphism at position -251 of the *IL-8* gene, 25.1% (57/227) of the individuals showed T/T homozygosity, 50.7% (115/227) showed T/A heterozygosity and 24.2% (55/227) showed A/A homozygosity. For polymorphism at position -819 of the *IL-10* gene, 47.1% (107/227) of the subjects exhibited the C/C genotype, 43.2% (98/227) the C/T genotype and 9.7% (22/227) the T/T genotype. Regarding polymorphism at position -592 of the *IL-10* gene, C/C homozygosity was found in 45.4% (103/227) of the patients, C/A heterozygosity in 45.4% (103/227) and A/A homozygosity in 9.2% (21/227).

According to Table 1, which provides the allelic and genotypic frequencies of *IL-6, IL-8* and *IL-10* gene polymorphisms in *H. pylori* positive and negative patients, only the presence of the A allele at position -251 of the *IL-8* gene was significantly associated with *H. pylori* infection ($p = 0.039$).

The distribution of the alleles and genotypes of *IL-6, IL-8* and *IL-10* gene polymorphisms in *H. pylori* positive and negative patients with normal gastric mucosa, gastritis, peptic ulcer disease and gastric carcinoma are displayed in Table 2. *H. pylori* positive subjects and carriers of the A/A genotype at *IL-8* gene position -251 showed an increased risk of peptic ulcer disease (PR = 2.08 and $_{95\%}$CI = 1.03-4.18). Additionally, carriers of the T allele at position -819 and the A allele at position -592 of *IL-10* have a higher risk of peptic ulcer disease in the presence of *H. pylori* infection (PR = 1.24 and $_{95\%}$CI = 1.01-1.53 for both alleles). No other association was

found between the genetic polymorphisms studied and the risk of gastroduodenal diseases.

## Discussion

Allelic variants in cytokine genes have been shown to influence gene expression and susceptibility to infectious diseases.[26] Naito et al.[27] showed that IL-8 levels in A allele carriers of the *IL-8* T-251A polymorphism were slightly higher than those in subjects with the T/T genotype. In this study, unlike some other 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), suggesting that this allele is related with susceptibility to *H. pylori* infection and its persistence.[13,28]

*H. pylori*-induced gastric mucosal inflammation is mediated by an array of pro- and anti-inflammatory cytokines.[5] In general, studies have shown that this inflammation is exacerbated in patients with high-producing alleles of pro-inflammatory cytokines and low-producing alleles of anti-inflammatory cytokines, which results in a higher risk of peptic ulcer or gastric carcinoma.[3] An ulcer occurs because of a disequilibrium between defensive mucosa-protective factors and aggressive injurious factors, and carcinogenesis occurs due to the accumulation of genetic alterations and the dysfunction of cellular mechanisms that normally maintain human genome integrity.[29,30]

Regarding peptic ulcer disease is important to register, in agreement to the results of other researchers, that we also observed that *H. pylori* positive patients and carriers of the A/A genotype at position -251 of the *IL-8* gene have an increased risk of this disorder.[6,31] Allele A is associated with higher IL-8 production and

consequently with increased mucosal injury due to activation and recruitment of neutrophils in response to infection by *H. pylori*.[32,33] However, unlike some authors who reported that *IL-10* gene polymorphisms are not associated with peptic ulcer, we arrived at the opposite conclusion.[25,34,35] After analyzing gastric biopsy samples from 227 patients, we found that carriers of the T allele at position -819 and the A allele at position -592 of *IL-10* have a higher risk of peptic ulcer disease in the presence of *H. pylori* infection. These two alleles are related to decreased IL-10 expression, which stimulates the pro-inflammatory response.[1]

Concerning gastric carcinoma, similar to Savage et al.,[36] Alpízar-Alpízar et al.,[37] and Savage et al.,[38], we also found no relationship between *IL-6, IL-8* and *IL-10* gene polymorphism and a higher risk of this pathology, despite other authors have reported to the contrary.[32,34,39]

These contradictory results found may be related to genetic and ethnic differences in the populations and also to differences in the study design, the patients' age at diagnosis, and their dietary habits. Since, the magnitude and direction of the inflammatory response is directed by host genetic factors interacting with environmental exposures.[40]

In conclusion, we observed that polymorphisms in the *IL-8* and *IL-10* genes were biologically important in the pathogenesis of peptic ulcer disease but not of gastric carcinoma in patients infected with *H. pylori*. It is likely that host genetic polymorphisms influence the course and/or gravity of gastric disease, but the extent to which they do so varies greatly among individuals/populations. A better understanding of these differences could determine the clinical significance of SNP profiles within the context of gastroduodenal disorders related to *H. pylori.*

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**Table 1.** Frequencies of the polymorphisms of *IL-6, IL-8* and *IL-10* genes in patients *H. pylori* positive and negative.

| | ***H. pylori* positive patients N (%)** | ***H. pylori* negative patients N (%)** | **PR ($_{95\%}$CI)** |
|---|---|---|---|
| **Alleles ➡ *IL-6* (-174)** | | | ***p* = 0.301[a]** |
| G | 218 (72.2%) | 114 (75.0%) | 1.0 |
| C | 84 (27.8%) | 38 (25.0%) | 1.05 (0.91-1.21) |
| **Genotypes ➡*IL-6* (-174)** | | | ***p* = 0.486[b]** |
| G/G | 79 (52.3%) | 45 (59.2%) | 1.0 |
| G/C | 60 (39.7%) | 24 (31.6%) | 1.12 (0.93-1.36) |
| C/C | 12 (8.0%) | 7 (9.2%) | 0.99 (0.69-1.43) |
| **Alleles ➡ *IL-8* (-251)** | | | ***p* = 0.039[a]** |
| T | 143 (47.4%) | 86 (56.6%) | 1.0 |
| A | 159 (52.6%) | 66 (43.4%) | 1.13 (0.99-1.29) |
| **Genotypes ➡*IL-8* (-251)** | | | ***p* = 0.139[b]** |
| T/T | 32 (21.2%) | 25 (32.9%) | 1.0 |
| T/A | 79 (52.3%) | 36 (47.4%) | 1.22 (0.94-1.59) |
| A/A | 40 (26.5%) | 15 (19.7%) | 1.30 (0.98-1.72) |
| **Alleles ➡ *IL-10* (-819)** | | | ***p* = 0.502[a]** |
| C | 208 (68.9%) | 104 (68.4%) | 1.0 |
| T | 94 (31.1%) | 48 (31.6%) | 0.99 (0.86-1.14) |
| **Genotypes ➡ *IL-10* (-819)** | | | ***p* = 0.941[b]** |
| C/C | 72 (47.7%) | 35 (46.1%) | 1.0 |
| C/T | 64 (42.4%) | 34 (44.7%) | 0.97 (0.80-1.18) |
| T/T | 15 (9.9%) | 7 (9.2%) | 1.01 (0.74-1.39) |
| **Alleles ➡ *IL-10* (-592)** | | | ***p* = 0.498[a]** |
| C | 205 (67.9%) | 104 (68.4%) | 1.0 |
| A | 97 (32.1%) | 48 (31.6%) | 1.01 (0.88-1.16) |
| **Genotypes ➡ *IL-10* (-592)** | | | ***p* = 0.989[b]** |
| C/C | 68 (45.0%) | 35 (46.1%) | 1.0 |
| C/A | 69 (45.7%) | 34 (44.7%) | 1.01 (0.84-1.23) |
| A/A | 14 (9.3%) | 7 (9.2%) | 1.01 (0.72-1.41) |

PR: Prevalence ratio; $_{95\%}$CI: 95% confidence interval

[a] *P*-values were determined by Fisher´s Exact Test

[b] *P*-values were determined by Chi-Squared Test

**Table 2.** Frequencies of the polymorphisms of *IL-6, IL-8* and *IL-10* genes in patients *H. pylori* positive and negative with normal gastric mucosa and with different gastric disorders.

| | **Normal Gastric Mucosa** | | | **Gastritis** | | | **Peptic Ulcer Disease** | | | **Gastric Carcinoma** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *H. pylori* positive patients N (%) | *H. pylori* negative patients N (%) | **PR ($_{95\%}$CI)** | *H. pylori* positive patients N (%) | *H. pylori* negative patients N (%) | **PR ($_{95\%}$CI)** | *H. pylori* positive patients N (%) | *H. pylori* negative patients N (%) | **PR ($_{95\%}$CI)** | *H. pylori* positive patients N (%) | *H. pylori* negative patients N (%) | **PR ($_{95\%}$CI)** |
| **Alleles *IL-6* (-174)** | | | ***p* = 0.335**[b] | | | ***p* = 0.826**[b] | | | ***p* = 0.655**[b] | | | |
| G | 34 (73.9%) | 25 (83.3%) | 1.0 | 122 (70.9%) | 65 (72.2%) | 1.0 | 52 (70.3%) | 18 (75.0%) | 1.0 | 10 (100.0%) | 6 (75.0%) | - |
| C | 12 (26.1%) | 5 (16.7%) | 1.22 (0.84-1.79) | 50 (29.1%) | 25 (27.8%) | 1.02 (0.84-1.24) | 22 (29.7%) | 6 (25.0%) | 1.06 (0.83-1.34) | - | 2 (25.0%) | - |
| **Genotypes *IL-6* (-174)** | | | ***p* = 0.328**[b] | | | ***p* = 0.908**[b] | | | ***p* = 0.643**[b] | | | |
| G/G | 12 (52.2%) | 11 (73.3%) | 1.0 | 45 (52.3%) | 25 (55.6%) | 1.0 | 17 (46.0%) | 7 (58.3%) | 1.0 | 5 (100.0%) | 2 (50.0%) | - |
| G/C | 10 (43.5%) | 3 (20.0%) | 1.47 (0.90-2.41) | 32 (37.2%) | 15 (33.3%) | 1.06 (0.81-1.38) | 18 (48.6%) | 4 (33.3%) | 1.16 (0.84-1.60) | - | 2 (50.0%) | - |
| C/C | 1 (4.3%) | 1 (6.7%) | 0.96 (0.23-4.05) | 9 (10.5%) | 5 (11.1%) | 1.0 (0.65-1.53) | 2 (5.4%) | 1 (8.4%) | 0.94 (0.41-2.18) | - | - | - |
| **Alleles *IL-8* (-251)** | | | ***p* = 0.253**[b] | | | ***p* = 0.304**[b] | | | ***p* = 0.350**[b] | | | ***p* = 0.681**[a] |
| T | 23 (50.0%) | 19 (63.3%) | 1.0 | 86 (50.0%) | 51 (56.7%) | 1.0 | 29 (39.2%) | 12 (50.0%) | 1.0 | 5 (50.0%) | 4 (50.0%) | 1.0 |
| A | 23 (50.0%) | 11 (36.7%) | 1.24 (0.86-1.77) | 86 (50.0%) | 39 (43.3%) | 1.10 (0.92-1.31) | 45 (60.8%) | 12 (50.0%) | 1.12 (0.88-1.42) | 5 (50.0%) | 4 (50.0%) | 1.00 (0.44-2.29) |
| **Genotypes *IL-8* (-251)** | | | ***p* = 0.458**[b] | | | ***p* = 0.465**[b] | | | ***p* = 0.482**[b] | | | ***p* = 0.757**[a] |
| T/T | 5 (21.7%) | 6 (40.0%) | 1.0 | 20 (23.3%) | 15 (33.3%) | 1.0 | 5 (13.5%) | 2 (16.7%) | 1.0 | 2 (40.0%) | 2 (50.0%) | 1.0 |
| T/A | 13 (56.6%) | 7 (46.7%) | 1.43 (0.69-2.95) | 46 (53.4%) | 21 (46.7%) | 1.20 (0.86-1.67) | 19 (51.4%) | 8 (66.6%) | 0.99 (0.58-1.67) | 1 (20.0%) | - | - |
| A/A | 5 (21.7%) | 2 (13.3%) | 1.57 (0.71-3.49) | 20 (23.3%) | 9 (20.0%) | 1.21 (0.83-1.76) | 13 (35.1%) | 2 (16.7%) | 2.08 (1.03-4.18) | 2 (40.0%) | 2 (50.0%) | 1.0 (0.25-4.0) |
| **Alleles *IL-10* (-819)** | | | ***p* = 0.059**[b] | | | ***p* = 0.721**[b] | | | ***p* = 0.069**[b] | | | |
| C | 30 (65.2%) | 13 (43.3%) | 1.0 | 124 (72.1%) | 63 (70.0%) | 1.0 | 47 (63.5%) | 20 (83.3%) | 1.0 | 7 (70.0%) | 8 (100.0%) | - |
| T | 16 (34.8%) | 17 (56.7%) | 0.69 (0.46-1.04) | 48 (27.9%) | 27 (30.0%) | 0.97 (0.79-1.18) | 27 (36.5%) | 4 (16.7%) | 1.24 (1.01-1.53) | 3 (30.0%) | - | - |
| **Genotypes *IL-10* (-819)** | | | ***p* = 0.132**[b] | | | ***p* = 0.730**[b] | | | ***p* = 0.241**[b] | | | |
| C/C | 9 (39.1%) | 2 (13.3%) | 1.0 | 45 (52.3%) | 21 (46.7%) | 1.0 | 15 (40.5%) | 8 (66.7%) | 1.0 | 3 (60.0%) | 4 (100.0%) | - |
| C/T | 12 (52.2%) | 9 (60.0%) | 0.70 (0.44-1.11) | 34 (39.5%) | 21 (46.7%) | 0.91 (0.70-1.18) | 17 (46.0%) | 4 (33.3%) | 1.24 (0.86-1.79) | 1 (20.0%) | - | - |
| T/T | 2 (8.7%) | 4 (26.7%) | 0.41 (0.13-1.31) | 7 (8.2%) | 3 (6.6%) | 1.03 (0.66-1.59) | 5 (13.5%) | - | - | 1 (20.0%) | - | - |
| **Alleles *IL-10* (-592)** | | | ***p* = 0.059**[b] | | | ***p* = 0.953**[b] | | | ***p* = 0.069**[b] | | | |
| C | 30 (65.2%) | 13 (43.3%) | 1.0 | 121 (70.3%) | 63 (70.0%) | 1.0 | 47 (63.5%) | 20 (83.3%) | 1.0 | 7 (70.0%) | 8 (100.0%) | - |
| A | 16 (34.8%) | 17 (56.7%) | 0.69 (0.46-1.04) | 51 (29.7%) | 27 (30.0%) | 0.99 (0.82-1.20) | 27 (36.5%) | 4 (16.7%) | 1.24 (1.01-1.53) | 3 (30.0%) | - | - |
| **Genotypes *IL-10* (-592)** | | | ***p* = 0.132**[b] | | | ***p* = 0.989**[b] | | | ***p* = 0.241**[b] | | | |
| C/C | 9 (39.1%) | 2 (13.3%) | 1.0 | 41 (47.7%) | 21 (46.7%) | 1.0 | 15 (40.5%) | 8 (66.7%) | 1.0 | 3 (60.0%) | 4 (100.0%) | - |
| C/A | 12 (52.2%) | 9 (60.0%) | 0.70 (0.44-1.11) | 39 (45.3%) | 21 (46.7%) | 0.98 (0.76-1.27) | 17 (46.0%) | 4 (33.3%) | 1.24 (0.86-1.79) | 1 (20.0%) | - | - |
| A/A | 2 (8.7%) | 4 (26.7%) | 0.41 (0.13-1.31) | 6 (7.0%) | 3 (6.6%) | 1.01 (0.61-1.65) | 5 (13.5%) | - | - | 1 (20.0%) | - | - |

PR: Prevalence ratio; $_{95\%}$CI: 95% confidence interval

[a] *P*-values were determined by Fisher´s Exact Test

[b] *P*-values were determined by Chi-Squared Test

# 4 CONCLUSÃO GERAL

Os dados obtidos no presente estudo evidenciaram que a aglomeração familiar e os polimorfismos nas regiões promotoras dos genes que codificam a *IL-1B* e a *IL-8* estão significativamente associados à infecção pelo *H. pylori.* Além disso, os pacientes *H. pylori* positivos e portadores dos genótipos T/T na posição -511 e C/C na posição -31 da *IL-1B* apresentaram um elevado risco de gastrite. Outra constatação relevante foi a de que indivíduos com o genótipo A/A na posição -251 da *IL-8* e com os alelos T na posição -819 e A na posição -592 de *IL-10* possuem um maior risco de doença ulcerosa péptica na presença da infecção pelo *H. pylori.* Almeja-se que os resultados alcançados neste trabalho possam, em breve, contribuir para uma menor incidência de infecção por *H. pylori* no Brasil, bem como permitir a identificação precoce dos pacientes infectados pelo *H. pylori* com elevado risco de desenvolverem desordens gástricas mais severas, de modo a viabilizar uma intervenção terapêutica mais apropriada e eficaz ao paciente. O conhecimento das bases moleculares do hospedeiro e do microrganismo possibilita o desenvolvimento de testes moleculares, os quais poderão ser aplicados em prol de um correto prognóstico das patologias gástricas.

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