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Helicobacter pylori oipA virulence gene as a molecular marker of severe gastropathies

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HIGHLIGHTS

- Evidence points to an association between the *H. pylori oipA* gene and gastropathies.
- There is a high prevalence of *H. pylori* infection with a relevant percentage of *oipA+* strains.
- More severe gastropathies were observed in those infected with *H. pylori oipA+* strains.

ABSTRACT – Background – *Helicobacter pylori* is an etiologic agent of gastroduodenal diseases. The microorganism, considered a type I carcinogen, affects about 50% of the global population. *H. pylori* virulence factors are determinant for the clinical outcome of the infection. The *outer inflammatory protein A* (*oipA*) gene encodes an outer membrane adhesin and is related to severe gastropathies, such as gastric cancer. **Objective –** The aim of this study was to evaluate the association of the *oipA* gene with the severity of gastroduodenal diseases in dyspeptic patients in region Central Brazil. **Methods –** The polymerase chain reaction (PCR) was used to determine the presence of *H. pylori*. Samples positives were used for molecular screening of the *oipA* gene. Gastropathies were categorized as non-severe and severe diseases. **Results –** Approximately 68% of patients had *H. pylori* and 36% were infected with *H. pylori oipA+* strains. Infection was significantly associated in patients aged over 44 years (P=0.004). However, there was no association between *oipA* and patients' age (P=0.89). Approximately 46% of patients infected with *oipA+* strains had some severe illness. Gastric adenocarcinoma was the most frequent severe gastropathy. The *H. pylori oipA* genotype was inversely associated with the severity of gastroduodenal diseases (OR=0.247, 95%CI: 0.0804–0.7149 and P=0.007). **Conclusion –** The characterization of possible molecular markers will contribute to personalized medicine, impacting the prognosis of patients.

Keywords – Bacterial gene; bacterial adhesion; bacterial outer membrane proteins.

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INTRODUCTION

Helicobacter pylori is a gram-negative, spiral, flagellate and microaerophilic bacterium. This microorganism is cosmopolitan and it is estimated that about 50% of the world population is infected[1,2]. The prevalence of *H. pylori* is associated with socioeconomic factors and hygiene conditions. In this context, higher infection rates are observed in developing countries[3]. In Brazil, the prevalence of infection varies from 31.7% to 91%, depending on the socioeconomic conditions of the different regions[4,5].

Infection with *H. pylori* can lead to the development of non-severe gastric diseases, such as duodenitis, esophagitis, gastritis, and ulcers, or severe, such as gastric atrophy, intestinal metaplasia, and adenocarcinoma. Because of its role in the pathogenesis of gastric cancer, the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) classifies this microorganism as a type I carcinogen[6-11].

The clinical outcome of the infection depends on the imbalance of the parasite-host relationship. *H. pylori* presents high genetic heterogeneity, and the different virulence factors are determinants for the development of severe diseases during the period of infection of the microorganism. Several virulence genes, such as *oipA*, may be associated with the severity of gastropathies[12,13].

The *oipA* gene encodes the *outer inflammatory protein A* (o*ipA*), an outer membrane protein that plays the role of adhesin. OipA acts on the phosphorylation of some signaling pathways that can induce inflammation along with pathways mediated by the *cag* pathogenic island (*cag*-PAI). The expression of *oipA* can stimulate several pro-inflammatory agents, mainly IL-8[14-16]. The induction of a more severe inflammatory response, through the activation of gene transcription, increases the risk for the development of peptic ulcer and gastric cancer[17].

Due to the high prevalence of infection and the well-established role in the etiology of severe gastroduodenal diseases, the characterization of possible molecular markers is extremely relevant for personalized medicine, impacting the diagnosis, prognosis and treatment of patients. In this sense, the aim of this study was to evaluate the association of the virulence gene *oipA* of *H. pylori* with the severity of gastroduodenal diseases in dyspeptic patients in central Brazil.

METHODS

Ethical considerations

This study was approved by the Research Ethics Committee of the *Hospital das Clínicas* of the Federal University of Goiás (HC/UFG), under protocol number 2.519.032 (CAAE: 83422017.7.0000.5078) and by the Research Ethics Committee of the Association de Goiás Combating Cancer in Goiás (ACCG), in Goiânia – Goiás, opinion no. 2.885.763 (CAAE: 95637418.3.3001.0031). All participants who agreed to participate in the study signed a Free and Informed Consent Form (FICT).

Population and samples

A total of 156 dyspeptic patients, aged between 18 and 75 years, confirmed by upper digestive endoscopy, were recruited for this study. Exclusion criteria were patients who used proton pump inhibitors, histamine-2 receptor blockers, or immunosuppressants/antibiotics. In addition, patients with active gastrointestinal bleeding, pregnant, lactating, or those who could not undergo endoscopy were excluded. One patient did not present the signed FICT and was excluded from this research. A total of 155 patients (117 from HC/UFG and 38 from ACCG) were included in this study, 47 (30.3%) male and 108 (69.7%) female. Regarding age, patients were categorized into two groups: aged 44 years or less and aged over 44 years, of which 109 (70.3%) were aged over 44 years.

Samples were collected by a gastroenterologist in accordance with the recommendations of the IV Brazilian Consensus on *H. pylori* infection. During endoscopy, two fragments were obtained from the antrum and two from the gastric body[17]. Tumor samples collected from patients with gastric cancer were obtained at the time of gastrectomy[18]. Two samples (one from the antrum and the other from the gastric body) were sent to the Clinical Pathology laboratory at HC/UFG for histopathological analysis. The other two samples were transported under refrigeration and stored at -20°C at the *Núcleo de Estudos da Helicobacter pylori* (NEHP), located at the Federal

University of Goiás. These samples were subjected to molecular analysis, in accordance with previous studies published by NEHP[19,20].

Histology

Samples obtained from gastric mucosal biopsies were fixed in 10% buffered formalin and stained with hematoxylin-eosin and Giemsa. The Sydney System was used to classify the histopathological parameters[21].

DNA extraction from biopsies

For DNA extraction, the protocol provided by the manufacturer Kit QIAamp DNA Mini Kit® (Qiagen, Valencia, CA, United States of America or USA) was used. The quantification and purity analysis of the genomic DNA was performed using optical density in a spectrophotometer (NanoDrop® ND-1000 UV-Vis).

Amplification of *H. pylori* DNA and the *oipA* virulence gene

The extracted DNA was used for the molecular screening of *H. pylori* infection by amplifying a fragment of the constitutive ribosomal 16S rRNA gene (*hpx*) using the polymerase chain reaction (PCR). The *hpx*-positive samples were then subjected to *oipA* gene detection.

Gene amplification (16S rRNA and *oipA*) was performed using the Amplitherm® thermocycler. Each reaction consisted of 0.5 µL of Taq-DNA polymerase (2.5 units), 5 µL of 10X CoralLoad PCR buffer (QIAamp, Qiagen) containing $MgCl_2$ (1.5 mM), 2 µL of dNTP (2.5 mM) and 33.5 µl milliQ water. After mixing, 5 µL of DNA from each sample (50 ng) were added, totaling a final volume of 50 µL. For each assay a negative and a positive control were used, with an *H. pylori* DNA aliquot kindly given by author Dr. Lucas Trevizani Rasmussen.

The specific oligonucleotides for each gene, the amplification conditions and the size of the fragments obtained are described in TABLE 1. PCRs will be performed using primers previously described in the literature[22,23].

PCR products were stained with BlueGreen nucleic acid (Lab Biotechnology®) and subjected to 2% agarose gel electrophoresis. Visualization was performed under ultraviolet (UV) light using a transilluminator.

Diagnosis of gastroduodenal diseases and severity criteria

Endoscopic and histopathological reports were used to segregate patients with gastroduodenal lesions according to severity. Lesions were categorized as non-severe (duodenitis, esophagitis, gastritis, and ulcers) and severe (gastric adenocarcinoma, gastric atrophy, and intestinal metaplasia)[24].

Data analysis

Data were analyzed using SPSS software (Statistical Package for the Social Sciences) version 17.0. The *P*-value was calculated using the chi-square and Fisher's exact tests, considering *P* values lower than 0.05 for statistical significance.

RESULTS

DNA extracted from gastric biopsies from 155 patients was used for molecular screening of *H. pylori*, through amplification of the 16S rRNA gene. A total of 67.7% of patients were positive for *H. pylori* infection. Positive samples were subjected to amplification of the 401 bp fragment of the *oipA* virulence gene. Of the total *H. pylori* positive samples, the *oipA*+ gene was detected in 36% (25/105), and 64% (80/105) of the samples were *oipA*-.

H. pylori infection was more prevalent in patients over 44 years of age (42.58%). The analysis of age groups of patients showed a significant association between infection and age (P=0.004). However, no association was found between the *oipA* genotype

TABLE 1. Sequence of primers used for the identification of *H. pylori* and *oipA*, amplification conditions and fragment size.

Gene	Oligonucleotides	Sequence (5' → 3')	Amplification conditions	bp
16S rRNA	*hpx1* *hpx2*	CTGGAGARACTAAGYCCCTC GAGGAATACTCATTGCAAGGCGA	94°C, 1 min; 59°C, 1 min; 72°C, 1 min (40 cycles)	150
oipA	HPO638F HPO638R	GTTTTTGATGCATGGGATTT GTGCATCTCTTATGGCTTT	94°C, 1 min; 56°C, 1 min; 72°C, 1 min (35 cycles)	401

R: GA (purine) and Y: TC (pyrimidine).

and the age of the patients (*P*=0.89). In this study, the presence of the infection was predominant in females (69.7%), but there was no significant association between the prevalence of the *oipA* genotype and the sex of the patients (*P*=0.95).

According to endoscopic and histopathological reports, patients infected with *H. pylori* had 88 clinical outcomes, 53 of which were non-severe and 35 were severe. The most prevalent non-severe gastroduodenal lesion was gastritis (32.33%), followed by esophagitis (5.26%) and duodenitis (1.5%). The most prevalent severe lesions were AdG (20.3%), gastric atrophy (3.76%) and metaplasia (2.25%). Regarding the presence of *oipA*, the most prevalent non-severe disease was gastritis (6.77%), and the most frequent severe disease was AdG (10.53%), as described in TABLE 2.

Descriptive analyzes showed that patients infected with *H. pylori* with *oipA*+ strains had 1.77 times more severe disease than those with non-severe disease. While, patients infected with *H. pylori oipA*- the presence of non-severe diseases was 2.31 times lower than those with severe diseases. However, the logistic regression model showed a significant inverse association between disease severity in patients infected with *H. pylori oipA*+, as the estimated odds ratio was less than 1 (OR: 0.247, CI: 0.080–0.714, *P*=0.007) (TABLE 3).

TABLE 2. Relationship between the presence of *oipA* and the severity of gastroduodenal diseases.

Type	Diseases	*oipA* +	*oipA* -
Severe	AdG	14 (10.53%)	13 (9.77%)
	Gastric atrophy	1 (0.75%)	4 (3.01%)
	Metaplasia	1 (0.75%)	2 (1.50%)
Not Severe	Esophagitis	0 (0%)	7 (5.26%)
	Duodenitis	0 (0%)	2 (1.50%)
	Gastritis	9 (6.77%)	34 (25.56%)
	Ulcer	0 (0%)	1 (0.75%)
Total	-	25 (28.40%)	63 (71.60%)

TABLE 3. Association between the presence of *oipA* and the severity of gastroduodenal diseases.

Diseases	*oipA* +	*oipA* -	OR	95%CI	*P*-value
Severe	16	19	0.247	0.0804 - 0.7149	0.007
Not Severe	9	44			

OR: Odds ratio. CI: Confidence interval.

DISCUSSION

Higher rates of *H. pylori* infection are reported in developing countries. In our study, the prevalence of infection was 67.7%. These data corroborate with studies conducted in Iran and Peru, where the rates were 76% and 62.9%[25]. On the other hand, a lower prevalence was found in Southeastern Brazil, with a proportion of 31%[4]. These differences can be attributed to socioeconomic conditions or to the different diagnostic methods used in the study.

Females were more affected by *H. pylori* infection, despite this, the presence of the *oipA* gene was not associated with the sex of the patients. In a study carried out in Iran with 86 patients, no significant difference was observed in the prevalence of the *oipA* genotype between men and women[26]. The different prevalence of *H. pylori* infection between the sexes can be explained by factors related to lifestyle, such as smoking and alcohol consumption. These factors favor the infection and progression of gastric diseases and are more frequently associated with males[27,28]. Furthermore, the characteristic heterogeneity of the *H. pylori* genome may explain the differences in the detection of the *oipA* gene in both sexes.

In this study, infection by the microorganism was more prevalent in patients over 44 years of age (70,3%). The age range of patients in adulthood was associated with *H. pylori* infection. A study carried out in Canada with 934 patients also showed a predominance of infection in adult patients[29]. On the other hand, Tran et al. found no association between age and *H. pylori* infection in the Vietnamese population[30]. In adults, there is a longer time of exposure of the microorganism throughout life. Furthermore, the levels of inflammation and ulceration are significantly higher in this age group when compared to children[31]. These factors may justify a higher prevalence of infection in adulthood.

In our findings, the *oipA*+ genotype of *H. pylori* was not associated with the age of the patients. This data corroborates the study carried out in Bulgaria, where the *oipA* gene was not associated with age[32]. Other virulence genes that are more characterized in *H. pylori*, such as the *cagA* gene, were not associated with any age group of patients. In contrast, the *vacA s1/s2* gene was significantly associated with the

age group of 31 to 40 years in a study on the Chinese population[33]. Age is a determining risk factor for the development of gastropathies, as patients are exposed to a longer period of tissue damage resulting from the virulence of molecular markers of the microorganism.

The *H. pylori oipA* gene was detected in 36% of dyspeptic patients. Relatively low rate compared to other countries such as Iran, Tunisia, Costa Rica and China, where the *oipA* gene was detected in 55%, 90.8%, 97% and 100% of samples, respectively[22,34-37]. The host's immune response and lifestyle, as well as the genetic polymorphism of *H. pylori* strains, may explain the different prevalences of *oipA* in these regions.

H. pylori oipA+ strains are associated with different severe and non-severe gastroduodenal diseases[17]. In our research, the most prevalent non-severe gastroduodenal lesion in patients infected with *H. pylori oipA*+ strains was gastritis. On the other hand, the most diagnosed severe lesion in patients with *H. pylori oipA*+ strains was gastric adenocarcinoma. A study carried out in the capital of Iran showed that the *oipA* genotype was more common in patients with gastritis than in patients with gastric cancer[38]. In Colombia and the USA the *oipA* gene was significantly associated with duodenal ulcer, gastric cancer, high *H. pylori* density and severe neutrophil infiltration. The combination of these factors can lead to increased mucosal inflammation, which represents an important risk factor for the severity of gastropathies[39-41].

In the present study, the descriptive analysis revealed that the presence of severe diseases in individuals infected with *H. pylori oipA*+ strains was approximately twice as high as individuals with non-severe diseases, that is, patients positive for *oipA* tend to develop more severe diseases when compared to to *H. pylori oipA*- patients. However, the *oipA*+ gene was inversely associated with the severity of gastroduodenal lesions. In a study conducted in Japan, the *oipA* gene was also associated with an increased risk of gastric cancer[42]. The functional status of the *oipA* gene of *H. pylori* allows better adhesion of the bacteria to the gastric epithelium, stimulating greater production of IL-8 and, consequently, tissue damage that favors the development of gastric cancer, especially when *oipA* is associated with other genes, such as *vacA* and *cagA*[17,43]. However, a study in Iran found a strong association of the *H. pylori oipA* genotype for ulcer risk and a significant reverse association with gastric cancer[44].

Clinical outcomes resulting from infection are due to the association of numerous virulence genes. A limitation of this study was the isolated analysis of the *oipA* gene, in addition to the lack of data on the functional status of this virulence gene. Other studies should be performed to determine the mechanisms associated with the development of gastropathies in patients infected with *H. pylori oipA*+ strains.

CONCLUSION

The *H. pylori oipA* genotype is an important pathogenicity marker and has been associated with the severity of gastroduodenal diseases. In this study, the prevalence of infection was 67.7%, and the *oipA* gene was detected in 36% of the patients. Patient's age and sex were not associated with *H. pylori oipA*+ strains. New studies will make it possible to evaluate this genotype associated with other virulence factors of *H. pylori* and the severity of gastric diseases. The molecular characterization of circulating strains in the region will contribute to personalized medicine, impacting the diagnosis, prognosis, and treatment of patients.

Authors' contribution

Maciel DN: study design, sample collection and processing, article writing and revision. Silva LLL: study design and sample processing. Assunção LP: study design and statistical analysis of data. Rasmussen LT: study design, article review. Barbosa MS: orientation, study design, revision, writing and correction of the article.

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RESUMO – Contexto – *Helicobacter pylori* é um agente etiológico de doenças gastroduodenais. O microrganismo, considerado cancerígeno tipo I, afeta cerca de 50% da população mundial. Os fatores de virulência do *H. pylori* são determinantes para o desfecho clínico da infecção. O gene da *proteína inflamatória externa A* (*oipA*) codifica uma adesina da membrana externa e está relacionado a gastropatias severas, como o câncer gástrico. **Objetivo –** O objetivo deste estudo foi avaliar a associação do gene *oipA* com a gravidade das doenças gastroduodenais em pacientes dispépticos na região Brasil Central. **Métodos –** A reação em cadeia da polimerase (PCR) foi utilizada para determinar a presença de *H. pylori*. Amostras positivas foram utilizadas para triagem molecular do gene *oipA*. As gastropatias foram categorizadas como doenças não severas e severas. **Resultados –** Aproximadamente 68% dos pacientes apresentaram *H. pylori* e 36% estavam infectados com cepas *H. pylori oipA*+. A infecção foi significativamente associada em pacientes com idade superior a 44 anos (P=0,004). No entanto, não houve associação entre *oipA* e a idade dos pacientes (P=0,89). Aproximadamente 46% dos pacientes infectados com cepas *oipA*+ tiveram alguma doença severa. O adenocarcinoma gástrico foi a gastropatia severa mais frequente. O genótipo *oipA* de *H. pylori* foi inversamente associado à gravidade das doenças gastroduodenais (OR=0,247, IC95%: 0,0804–0,7149 P=0,007). **Conclusão –** A caracterização de possíveis marcadores moleculares contribuirá para a medicina personalizada, impactando no prognóstico dos pacientes.

Palavras-chave – Gene bacteriano; adesão bacteriana; proteínas da membrana externa bacteriana.

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