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*Helicobacter pylori vac*A virulence gene analysis and association with clinical and histopathological aspects in dyspeptic patients

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ABSTRACT. *Helicobacter pylori* is a Gram-negative oncobacterium that affects more than 50% of the world population. The differing clinical outcomes resulting from *H. pylori* infection are the result of host-pathogen interactions. The vacuolating cytotoxin A (*vac*A) gene is considered an important virulence factor in *H. pylori* as it has been associated with gastric complications. The aim of this study was to detect the *H. pylori vac*A genotype and determine if it is associated with gastropathies. A total of 117 gastric biopsy samples were collected from dyspeptic patients in central Brazil. The detection of the microorganism was performed using specific primers for the 16S rRNA and *vac*A genes. Subsequently, gastric pathologies were classified as severe (atrophy, metaplasia and adenocarcinoma) and non-severe (esophagitis, duodenitis, gastritis and ulcers) based on histopathological examination. A total of 80 patients were *H. pylori* positive and (56%) of those were *vac*A positive. Infection with the positive *H. pylori vac*A strain was more prevalent in patients over the age of 45 years. Gastric diseases were diagnosed in 91% of patients infected with *H. pylori*. Gastritis was the most common histopathological finding in the positive (69%) and negative (70%) *vac*A groups (p=1.00). However,

there were no significant associations between *H. pylori vac*A-positive status, sociodemographic characteristics, and clinical outcomes. The *vac*A gene was not found to be a marker of the severity of gastric lesions in our study (OR: 0.70, P=0.67). Nevertheless, the *vac*A gene was found to have a high prevalence in *H. pylori* isolates from gastric lesions in these Brazilian patients. Given this high frequency and what is known about this virulence factor, it would be useful to evaluate the interaction of *vac*A with other genes that affect the severity of gastric lesions.

Key words: Oncobacteria; Virulence factors; Vacuolating Cytotoxin A; Gastropathies; Personalized Medicine

INTRODUCTION

Helicobacter pylori is a Gram-negative, microaerophilic, flagellated, polymorphic bacterium that colonizes the human gastric epithelium (Marshall and Warren, 1984). Infection with this bacterium is closely associated with the development of gastropathies, such as chronic gastritis, peptic ulcer and gastric adenocarcinoma (AdG) (Sonnenberg and Genta, 2015; El Khadir et al., 2017; Venneman et al., 2018). This pathogen has a high degree of genomic plasticity among strains, due to high mutation and recombination rates (Vale and Lehours, 2018).

H. pylori infects more than 50% of the world population; in developing countries the prevalence can reach about 90% of the population. This high prevalence in emerging countries is associated with inadequate health and hygiene conditions (Li et al., 2023). In Brazil, in poor populations located in the north and northeast of the country, the prevalence rate is similar to that of Africa, with about 70-90% (Thorell et al., 2017; Basílio et al., 2018; Li et al., 2023).

Approximately 80% of stomach cancer cases are attributed to chronic *H. pylori* infection, a reason that led the International Agency for Research on Cancer (IARC) to classify the pathogen as class I carcinogen (IARC, 2014). This infection is treated with several different therapeutic regimens, the triple regimen being the first-line treatment. This treatment includes a proton pump inhibitor and a combination of two antibiotics, such as clarithromycin and amoxicillin or metronidazole, for a period of 14 days. However, due to the high prevalence of resistant multidrug *H. pylori* strains, this regimen has had reduced effectiveness (Coelho et al., 2018; Boyanova et al., 2019).

The different clinical outcomes resulting from *H. pylori* infection are the results of the interaction between environmental factors, host genetics and virulence factors of the microorganism (Roesler et al., 2014). Virulence factors allow for better adaptation of the pathogen and favors the development of infection. The virulence genes *cag*A and *vac*A are the most characterized and commonly used in the genotyping of *H. pylori* in clinical isolates (El Khadir et al., 2017; Akeel et al., 2019). However, evaluation of *H. pylori* strains in some countries, including Brazil, is still not common.

The *vac*A virulence gene codes for the VacA protein, which triggers cytoplasmic vacuolization in gastric cells, which induces the release of cytochrome C from mitochondria, promoting the pro-inflammatory response and apoptosis. In addition, VacA binds to the cell epithelium interacting with tyrosine phosphatase proteins, which increases the formation of pores in cell membranes (Roesler et al., 2014; Inagaki et al., 2017). This

gene is present in most strains of *H. pylori* and presents allelic diversity in three main regions, the s (signal), i (intermediate) and m (medium) regions. The combination of two different alleles from each region (s1, s2, i1, i2, m1, m2) gives a distinct ability to induce vacuolization in epithelial cells (Nicolescu, 2014; Inagaki et al., 2017). The *vac*A s1/m1 strains are vacuolizing, the *vac*A s2/m2 strains are not vacuolizing and some *vac*A s1/m2 strains are able to induce the formation of cellular vacuoles. Among these genotypes, *vac*A s1/m1 is considered the most virulent among *H. pylori* strains (Roesler et al., 2014; Inagaki et al., 2017).

Genetic diversity by recombination probably depends on the presence of co-infection with multiple strains, being more common in areas with endemic *H. pylori* infection (Thorell et al., 2017). This genetic variability of *H. pylori* is associated with various types of gastric complications and resistance to treatment. Along this line, the aim of this study was to detect the *H. pylori vac*A genotype and determine if it correlates with clinical outcomes and histopathological changes.

MATERIAL AND METHODS

Study population and ethical considerations

The cross-sectional study was carried out at a University Hospital in Goias, located in central Brazil. The study was approved by the Research Ethics Committee of the Federal University of Goias (UFG), under the opinion of no. 2.519.032. Inclusion criteria were dyspeptic patients, over 18 years of age who agreed to sign the Informed Consent Forms. Exclusion criteria were patients with gastrointestinal bleeding, pregnant, lactating, history of gastrectomy or who received antimicrobial and immunosuppressive therapy for less than four weeks and proton pump inhibitors for less than two weeks before collection.

Samples

The samples were collected according to the recommendations of the IV Brazilian Consensus on Helicobacter pylori infection (Coelho et al., 2018). A total of 117 gastric biopsy samples from patients undergoing upper gastrointestinal endoscopy were obtained. From each patient, four tissue fragments were collected, two from the antrum and two from the gastric body. The fragments were sent to the Núcleo de Estudo da Helicobacter pylori (NEHP) at UFG and to the pathology department of University Hospital, for molecular diagnosis and histopathological analysis, respectively.

DNA extraction and purification

DNA extraction was performed according to the protocol provided by the manufacturer KitQIamp DNA minikit® (Qiagen, Valencia, CA, United States). The concentration and purity of genomic DNA was determined by optical density in a spectrophotometer (NanoDrop® ND-1000 UV-Vis) and stored at -20 ° C.

DNA Amplification by Polymerase Chain Reaction (PCR)

Molecular detection of H. pylori was performed using the PCR technique. The microorganism was screened using the ribosomal 16S rRNA gene using a pair of hpx (sense) and hpx1 (antisense) oligonucleotides. Positive samples were submitted to amplification of the vacA virulence gene under the following conditions: 0.5 µL of Taq DNA Polymerase (2.5 units), 5 µL of 10x Buffer PCR containing $MgCl_2$ (1.5 mM), 2 µL (2.5 mM) of dNTP (deoxyribonucleic 5'-triphosphate), 2 µL of each oligonucleotide (vacASA and vacASca) (10 pmol), 33.5 µL of Milli-Q water and 5 µL (50 ng) of DNA, totaling 50 µL for each reaction. A positive control (strain ATCC 26695) and a negative control (water free of nucleases) were included in each reaction. The primers used, the amplification conditions and the amplicon size are described in Table 1. The PCR products were electrophoresed on a 2% agarose gel, stained with Bluegreen and visualized under an Ultraviolet Light (UV).

Table 1. Primers used, amplification conditions and size of the amplified fragment of the Helicobacter pylori 16S rRNA and vacA genes.

Gene	Primer	Sequence (5' → 3')	PCR Conditions	bp	Reference
16S rRNA	*hpx* *hpx1*	*Forward* CTGGAGARACTAAGYCCTCC *Reverse* GAGGAATACTCATTGCGA AGGCGA	94°C, 5min, 40 cycles: 94ºC, 1min/ 59°C, 1min/ 72°C, 1min/ 72°C 7min	150	(Scholte et al., 1997)
*vac*A	*SA* *Sca*	*Forward* ATGGAAATACAACAAACACAC *Reverse* CCTGARACCGTTCCTACAGC	94°C, 5min 35 cycles: 94°C, 45s/ 53°C, 45s/ 72°C, 45s/ 72° 7 min	286	(Van Doorn et al., 1998)

DNA sequencing

All vacA gene amplifcation products were purified with the Wizard SV Gel and PCR Clean-Up System (Promega, Madison WI, USA). DNA sequencing was performed using an ABI-PRISM BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Carlsbad, CA, USA) in an ABI-PRISM 3100 genetic analyzer (Applied Biosystems).

Sequencing was performed at least twice, using the same forward and reverse primers used in the PCR assays. All the sequences were compared with those listed on the Gen-Bank nucleotide and protein databases (National Center for Biotechnology Information, Bethesda, MD, USA), using the Blastn and Blastx algorithms, respectively.

Histopathological examination

All biopsies were submitted to histopathological examination to investigate the presence of the bacteria and tissue changes. The biopsies were fixed in 10% buffered formalin, cut and stained with Hematoxylin and Eosin (H&E) or Giemsa. The slides were analyzed according to the Sydney classification system (Dixon et al., 1996). Gastric pathologies were classified between severe (atrophy, metaplasia and adenocarcinoma) and

non-severe (esophagitis, duodenits, gastritis and ulcers) as described by Paredes-Osses et al. (2017) and Bellolio et al. (2018).

Statistical analysis

Fisher's exact test was used to verify the association between sociodemographic variables sex, clinical conditions and the presence of H. pylori infection and the vacA gene. Logistic regression was used to model and evaluate the association between the severity of gastric pathologies in individuals infected with H. pylori vacA positive. For statistical analysis, the software R® version 3.5.0 for Windows was used with the level of significance considered $\alpha = 0.05$.

RESULTS

A total of 117 gastric biopsy samples obtained from patients undergoing upper digestive endoscopy were analyzed; 92 were female and 25 were male. Among these patients 68% were positive for *H. pylori* and 56.0% had the *vac*A gene (Table 2). The sequencing was performed to confirm the results observed by PCR.

Table 2. Sociodemographic distribution, clinical outcome and association with *Helicobacter pylori*.

Variables	N (%)	***H. pylori* +** n (%)	***H. pylori* –** n (%)	**p value**
Total	117 (100)	80 (68.4)	37 (31.6)	
Sex				
Man	25 (21.4)	17 (21.2)	8 (21.6)	1.00
Women	92 (78.6)	63 (78.8)	29 (78.4)	
Age group				
18-24	10 (8.5)	9 (11.2)	1 (2.7)	
25-34	15 (12.8)	13 (16.3)	2 (5.4)	0.05
35-44	18 (15.4)	14 (17.5)	4 (10.8)	
≥45	74 (63.3)	44 (55.0)	30 (81.1)	
Clinical outcomes*				
Gastritis	80 (68.4)	55 (68.7)	25 (67.6)	1.00
Duodenitis	16 (13.7)	13 (16.3)	3 (8.1)	0.38
Esophagitis	16 (13.7)	11 (13.7)	5 (11.9)	1.00
Gastric Ulcer	4 (3.4)	1 (1.3)	3 (8.1)	0.09
Atrophy	13 (11.1)	6 (7.5)	7 (18.9)	0.11
Adenocarcinoma	2 (1.7)	2 (2.5)	0	1.00
Xanthelasma	1 (0.8)	1 (1.3)	0	1.00
Metaplasia	7 (6.0)	3 (3.7)	4 (10.8)	0.20
Normal	18 (15.4)	14 (17.5)	4 (10.8)	0.42
Severe gastric diseases				
No	98 (83.8)	70 (87.5)	28 (75.7)	0.11
Yes	19 (16.2)	10 (12.5)	9 (24.3)	
Symptomatology				
Asymptomatic	3 (2.6)	3 (3.8)	0	0.55
Symptomatic	114 (97.4)	77 (96.2)	37 (100.0)	
Erradication therapy				
Treated	47 (49.0)	29 (48.3)	20 (55.5)	0.53
Untreated	49 (51.0)	31 (51.7)	16 (44.5)	
Missing	18	20	1	

The distribution of the *vac*A gene by sex showed a female predominance (69%). The age of patients infected with *H. pylori vac*A positive ranged between 18 and 71 years and 48% of the patients were older than 45 years. The presence of the *vac*A genotype increased proportionally with the age of the patients. However, no significant difference was found between sex, age group and positive for *H. pylori vac*A infection (Table 3).

Table 3. Sociodemographic distribution, clinical outcome and association with the *vac*A gene in *Helicobacter pylori*.

Variables	N (%)	*vac*A + n (%)	*vac*A – n (%)	P value
Total	117 (100)	42 (56.0)	33 (44.0)	
Sex				
Man	25 (21.4)	13 (30.9)	4 (12.1)	0.09
Women	92 (78.6)	29 (69.1)	29 (87.9)	
Age group				
18-24	10 (8.5)	6 (14.3)	3 (9.1)	
25-34	15 (12.8)	6 (14.3)	5 (15.2)	0.45
35-44	18 (15.4)	10 (23.8)	4 (12.1)	
≥45	74 (63.3)	20 (47.6)	21 (63.6)	
Clinical outcomes*				
Gastritis	80 (68.4)	29 (69.0)	23 (69.7)	1.00
Duodenitis	16 (13.7)	7 (16.7)	6 (18.2)	1.00
Esophagitis	16 (13.7)	4 (9.5)	7 (21.2)	0.20
Gastric Ulcer	4 (3.4)	0	1 (3.0)	0.44
Atrophy	13 (11.1)	2 (4.8)	2 (6.1)	1.00
Adenocarcinoma	2 (1.7)	1 (2.4)	1 (3.0)	1.00
Xanthelasma	1 (0.8)	0	1 (3.0)	0.44
Metaplasia	7 (6.0)	1 (2.4)	1 (3.0)	1.00
Normal	18 (15.4)	8 (19.0)	5 (15.5)	0.76
Severe gastric diseases				
No	98 (83.8)	39 (92.9)	29 (87.9)	0.69
Yes	19 (16.2)	3 (7.1)	4 (12.1)	
Symptomatology				
Asymptomatic	3 (2.6)	2 (4.8)	1 (3.0)	1.00
Symptomatic	114 (97.4)	40 (95.2)	32 (97.0)	
Erradication therapy				
Treated	47 (49.0)	11 (36.7)	16 (57.1)	0.19
Untreated	49 (51.0)	19 (63.3)	12 (42.9)	
Missing	18	12	5	

A total of 52 clinical outcomes were detected in patients that were *H. pylori vac*A positive. Since the same patient could have more than one gastric lesion, the number of clinical outcomes was greater than the number of patients. The most prevalent non-severe gastric injury was gastritis (69%), followed by duodenitis (17%) and esophagitis (9.5%). In contrast, the most prevalent severe lesions were atrophy (4.8%), followed by metaplasia (2.4%) and adenocarcinoma (Table 3). Statistical analysis by logistic regression showed no significant relationship between severity of lesions, age, sex and *H. pylori* and *vac*A (Table 4).

Gastric biopsy samples from *H.pylori vac*A positive patients with severe and non-severe lesions were used for photomicrography (Figure 1). Photomicrographs 1A show normal mucosa and 1B, non-severe lesions compatible with gastritis. Figures 1C and 1D show intestinal metaplasia and adenocarcinoma (Figure E and F), considered severe lesions.

Table 4. Logistic regression analysis of the presence of *Helicobacter pylori*, *vac*A gene, age, sex and severe gastric diseases.

Variables	Severe gastric diseases		OR (95% IC)	AIC	BIC	P value
	No n (%)	Yes n (%)				
Sex						
Male	20 (20.4)	5 (26.3)	Ref	101.27	109.55	0.91
Female	78 (79.6)	14 (73.7)	0.94 (0.29 – 3.35)			
H. pylori						
No	28 (28.6)	9 (47.4)	Ref	100.29	108.58	0.32
Yes	70 (71.4)	10 (52.6)	0.58 (0.24 – 1.71)			
***vac*A**						
No	29 (42.6)	4 (57.1)	Ref	47.38	54.33	0.67
Yes	39 (57.4)	3 (42.9)	0.70 (0.12 – 3.64)			

AIC: Akaike Information Criterion and BIC: Bayesian Information Criterion.

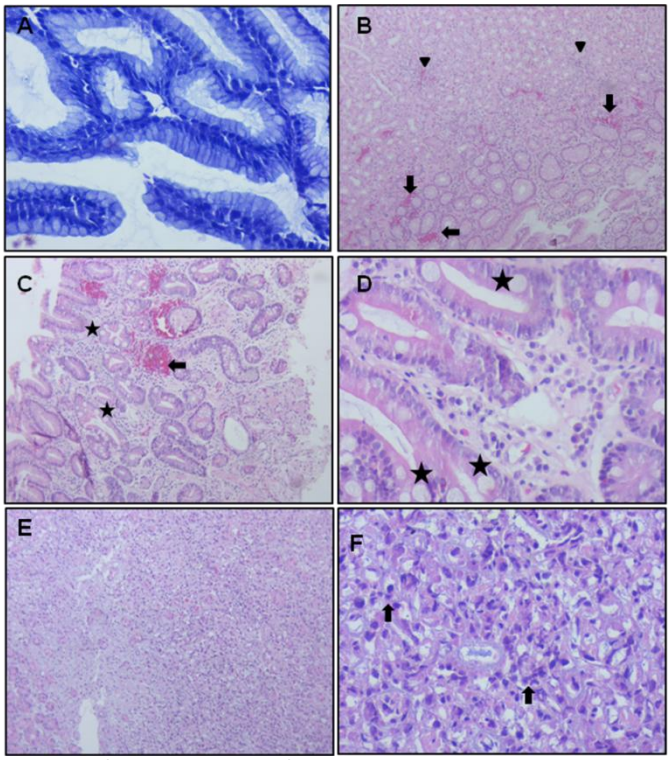

Figure 1. Photomicrographs of gastric biopsies of human tissue**. A:** gastric mucosa within normal standards, Giemsa, 40X. **B:** discrete chronic gastritis - gastric mucosa with discrete and multifocal inflammatory infiltrate (arrowhead) and moderate hyperemia (arrow). H&E, 10X**. C:** intestinal metaplasia - presence of plasmacytic infiltrate, deposition of fibrous tissue, moderate multifocal hemorrhage (arrow), presence of goblet cells (star), H&E, 10X. **D:** intestinal metaplasia, presence of goblet cells (star), diffuse and moderate inflammatory lymphoplasmacytic infiltrate, diffuse and moderate fibrosis, atrophy/absence of gastric glands. H&E, 40X. **E:** poorly differentiated adenocarcinoma presenting marked proliferation of gastric epithelial cells with loss of cell pattern and with characteristics of malignancy. H&E, 10X. **F:** marked proliferation of neoplastic gastric epithelial cells with little differentiation. Presence of cells with marked anisocariosis (arrow), cell pleomorphism, scarce eosinophilic cytoplasm and atypical mitosis. H&E, 40X.

The frequency of reporting gastrointestinal symptoms was high (97.4%) among the patients included in the study. Of the positive *H. pylori vac*A patients, 95.2% had symptoms. The main symptoms described by dyspeptic patients were gastralgia, heartburn, eructations, nausea, emesis and dysphagia. However, there was no significant difference between the variables (Table 2).

The 117 patients included in the study were classified into two groups: treated (49%) and untreated (51%). Of the total *H. pylori* positive patients, 48% underwent conventional *H. pylori* eradication treatment (Table 2). In contrast, about 37% of *H. pylori vac*A positive patients were treated with triple therapy. Most patients positive for *H. pylori vac*A (63%) did not receive eradication therapy (Table 3).

DISCUSSION

The *vac*A gene was detected in 56% of patients infected with *H. pylori*. This result is considered low when compared to studies carried out in the north, northeast and southeast regions of Brazil, which showed an average of 84.6%, 86.1% and 69.4% respectively (Rasmussen et al., 2012; Silva et al., 2013; Basílio et al., 2018; Bellolio et al., 2018). In other countries, such as Saudi Arabia (100%) (Akeel et al., 2019), Morocco (99%) (El Khadir et al., 2018) and China (95%) (Pinto-Ribeiro et al., 2016), higher rates of detection of the *vac*A gene have also been reported. The low detection rate of the *vac*A virulence gene in *H. pylori* strains found in our study can be attributed to bacterial genetic polymorphisms (Vale and Lehours, 2018).

In our study, most patients were female, which was also found in other investigations, though apparently women seek health services more frequently (Levorato et al., 2014; Ibrahim et al., 2018; Ferro et al., 2019). The search for health services facilitates early detection and, consequently, effective treatment, which may justify the greater prevalence of infection in males. In addition, studies show that men are more exposed to risk factors (Ferro et al., 2019). Although most patients in our study were female, there was no significant difference between sexes in *H. pylori vac*A positive infection.

The frequency of positive infection by *H. pylori vac*A was higher in adulthood, with a predominance from 45 years of age. Similar results were observed in the study by Alarcón et al.,(1999), who demonstrated that the detection of the *vac*A gene was proportional to the age of the patients. In Cuba, positive strains of *H. pylori vac*A were detected in 73.5% of patients over 40 years of age (Feliciano et al., 2015). Although the frequency of infection increased with age, there was no association, which corroborates other studies in which the *vac*A gene and advanced age were not associated (El Khadir et al., 2017; Akeel et al., 2019).

The association of a virulence factor can inhibit the action of another. A study carried out by Oliveira et al. (2021), in this same population from the central region of Brazil, identified the *cag*A gene in 80.5% of the population and found no association with the development of more serious gastric lesions. Recent studies suggest that the effects provoked by *cag*A in the host cell can neutralize those triggered by *vac*A. Similar to the effects of *vac*A, they also interfere with the action of the *cag*A gene. It is suggested that the antagonism between these genes allows *H. pylori* to regulate host cell responses without

causing serious cellular damage (Palframan et al., 2012). These findings explain our findings, in which no relationship was found with the severity of gastric and *vac*A lesions.

Sonnenberg and Genta (2015), in a study conducted in the United States, observed that throughout the life of patients in areas a high prevalence of *H. pylori*, the mucosa was altered in 50% and complications such as chronic active gastritis, gastric atrophy and intestinal metaplasia were significantly diagnosed. Notoriously, with increasing age, there is a change in the topographic distribution of *H. pylori* and expansion of the pyloric glands in the distal-proximal gastric direction. This modification is due to the strong affinity of the pathogen for the antrum, which is slightly acidic in relation to the body. Thus, the comorbidity of gastric diseases is significantly higher in the elderly population, due to the uniform distribution of the infection throughout the gastric mucosa (Sonnenberg and Genta, 2015; Shi et al., 2018).

The *vac*A gene was detected more frequently in patients with non-serious diseases such as gastritis and duodenitis. Despite the well-established role of this gene in the development of severe gastropathies, a hypothesis that may justify this finding is the polymorphism of the *vac*A gene and the interaction with other virulence genes of *H. pylori*. In addition, the persistence of the infection associated with lifestyle can also worsen the patient's condition, resulting in the development of serious disease at an early stage, such as stomach cancer (Venneman et al., 2018).

Pinto-Ribeiro et al. (2016) demonstrated in a case-control study conducted with patients diagnosed with chronic gastritis and gastric carcinoma, that Chinese infected with *H. pylori vac*A had a worse prognosis for gastric diseases. This evaluation allowed to identify that 95% of the patients infected with *H. pylori* presented the region s of the *vac*A gene, mainly of the most virulent allele *vac*A s1, detected in 91.2% of the cases.

All *H. pylori vac*A negative patients were dyspeptic and older (> 45 years). This fact may be due to the prolonged exposure time to harmful agents, such as the use of non-steroidal anti-inflammatory drugs, salt consumption, hot foods, fried foods, canned, smoking and alcohol consumption. It is also important to consider the low quality of sleep and the individual's occupation (Sonnenberg and Genta, 2015; Ferro et al., 2019; Huang et al., 2020; Shah et al., 2020).

In our study, 95% of *H. pylori vac*A positive patients were symptomatic. Among the symptoms, gastralgia was the most reported by patients. The mechanisms that cause different gastric symptoms are still not well understood. It is known that lesions in the gastric mucosa are the result of the action of bacterial toxins and an increase in the host's pro-inflammatory cytokines. Numerous studies have been carried out with symptomatic and asymptomatic patients infected with positive *H. pylori cag*A and *vac*A strains and have not found a significant relationship between the presence of these virulence genes and symptomatology (Nicolescu, 2014; Roesler et al., 2014; Chojnacki et al., 2019).

Mechanical stimuli and inflammatory mediators, such as serotonin are factors that can induce visceral pain (Mawe and Hoffman 2013, Chojnacki et al., 2019). A study in Poland found that expression of tryptophan hydroxylase (TpH1) was significantly higher in symptomatic positive *H. pylori* patients than in those without symptoms, which may indicate a direct induction of TpH1 by pro-inflammatory cytokines (Chojnacki et al., 2019). The reports of symptomatology are important and should be considered in the evaluation, although they are nonspecific.

In that study, 36.7% of patients infected with *H. pylori vac*A underwent first-line treatment. Different therapies for the eradication of *H. pylori* have been proposed and are used as one of the strategies for the prevention of gastric cancer (IARC 2014). Relapse of *H. pylori* in previously treated patients can occur due to recrudescence or reinfection (Vianna et al., 2016). Although triple therapy is considered the first-line treatment, this regimen has reduced effectiveness in several countries due to bacterial resistance (Samie et al., 2014). In 2017, World Health Organization (2017) issued a report on antibiotic-resistant pathogens, which are considered a priority for guiding research and development of new antimicrobial drugs. *H. pylori* ranks fifth on the list, classified as a high priority (World Health Organization 2017).

Studies carried out in Australia, Belgium, Brazil, France, the Netherlands, and Switzerland have found no association of *vac*A with antibiotic resistance (Van Doorn et al., 2001; Ecclissato et al 2002). Unlike studies carried out in Bulgaria where an association was found between resistance to clarithromycin and *vac*A genotypes (Boyanova et al.,2015). In Turkey, an association of *vac*A and *cag*A with resistance to clarithromycin and metronidazole has been demonstrated in children (Karabiber et al., 2014). Many *H. pylori vac*A positive resistant genotypes have been identified in samples of raw milk from bovine, ovine, caprine, buffalo and camel species (Ranjbar et al., 2018). Thus, these foods can be a vehicle for the propagation of resistant strains, which can result in the failure of the first-line therapy observed in this research.

CONCLUSION

In our study, the detection of the *vac*A gene was found to be low compared to other regions of Brazil. Females and patients over 45 years of age were more affected. Most *H. pylori vac*A positive patients had gastric lesions. Gastritis was the most common clinical outcome in dyspeptic patients. The presence of the isolated *vac*A gene was not considered a marker of the severity of gastric lesions in the present study. Additional studies are important to evaluate the interaction of *vac*A with other virulence genes that determine the severity of gastric lesions. The findings open perspectives for the development of personalized medicine in Brazil.

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ETHICAL APPROVAL

The study was conducted according to the ethical standards of the Research Ethics Committee of the University Hospital of the Federal University of Goias (HC/UFG), under the opinion number 2.519.032 and CAAE: 83422017.7.0000.5078, according to Resolution CNS / 196/96.

CONSENT TO PARTICIPATE

Informed consent was obtained from all individual participants included in the study.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study. The author Daniela Medeiros Milhomem Cardoso carried out the recruitment of participants. The standardization of PCR reactions was performed by the author Lucas Trevizani Rasmussen. Interviews with patients, construction and consistency of the database were performed by Amanda Ferreira Paes Landim Ramos, Jaqueline Correia Pontes Serra and Lucas Luiz de Lima Silva. Data analysis was performed by Amanda Ferreira Paes Landim Ramos and Mônica Santiago Barbosa. The analysis and interpretation of the histopathological examination slides was performed by Marina Pacheco Miguel. The first version of the manuscript was written by Jaqueline Correia Pontes Serra and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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