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Major Article

cagE as a biomarker of the pathogenicity of *Helicobacter pylori*

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ABSTRACT

Introduction: *Helicobacter pylori* infection is associated with gastro-duodenal diseases. Genes related to pathogenicity have been described for *H. pylori* and some of them appear to be associated with more severe clinical outcomes of the infection. The present study investigates the role of *cagE* as a pathogenicity biomarker of *H. pylori* compare it to *cagA, vacA, iceA* and *babA2* genes and correlate with endoscopic diagnoses. **Methods:** Were collected biopsy samples of 144 dyspeptic patients at the Hospital of the Federal University of Rio Grande, Rio Grande do Sul, Brazil. After collection, the samples were sent for histological examination, DNA extraction and detection of all putative pathogenicity genes by PCR. **Results:** Of the 144 patients undergoing endoscopy, 57 (39.6%) presented *H. pylori* by histological examination and PCR by detection of the *ureA* gene. Based on the endoscopic diagnoses, 45.6% (26/57) of the patients had erosive gastritis, while 54.4% (31/57) had enanthematous gastritis. The genes *cagA, cagE, vacAs1/m1, vacAs1/m2* and *iceA1* were related to erosive gastritis, while the genes *vacAs2/m2*, *iceA2* and *babA2* were associated to enanthematous gastritis. We found a statistically significant association between the presence of *cagE* and the endoscopic diagnosis. However, we detect no statistically significant association between the endoscopic diagnosis and the presence of *cagA, vacA, iceA* and *babA2,* although a biological association has been suggested. **Conclusions:** Thus, *cagE* could be a risk biomarker for gastric lesions and may contribute to a better evaluation of the *H. pylori* pathogenic potential and to the prognosis of infection evolution in the gastric mucosa.

Keywords: *Helicobacter pylori.* Pathogenicity genes. Endoscopic diagnosis.

INTRODUCTION

Helicobacter pylori, a microorganism adapted to colonize the gastric mucosa, is considered to be the main etiological agent of enanthematous gastritis (inflammation of the gastric epithelium with simple change mucosal), erosive gastritis (inflammation with loss of integrity of the epithelial lining, not exceeding the muscular layer of the mucosa) and also a risk factor for peptic ulcer and gastric cancer in humans[1,2]. Factors related to the genetic polymorphism of the host, the diversity of bacterial pathogenicity and the environment seem to be related to the broad clinical spectrum related to infection by *H. pylori*[3]. Several putative genes, such as *cagA, cagE, vacA, iceA* and *babA2*, have been identified and are likely to play an important role in the pathogenicity of the bacterium[4-8].

The *cag*-PAI is composed of approximately 31 genes, which are responsible for coding type IV secretion system components and inject effector molecules in the host cell. The presence of *cag-PAI* affects the inflammatory state of the gastric mucosa by polymorphonuclear cell infiltration and increases the production of interleukin-8 (IL-8)[1]. *cagA* gene (*cytotoxin associated gene A*) is considered to be the *cag-PAI* marker. *cagA* positive strains tend to be more pathogenic, produce more severe lesions of the epithelium and increase the expression of interleukin-1β and IL-8[9,10]. Another member of the *cag-PAI,* the *cagE* gene (*cytotoxin associated gene E*), is also related to an increased production of IL-8 in the gastric epithelial cells[11].

The *vacA* gene encodes the vacuolating cytotoxin that damages the gastric epithelial cells. It comprises two variable parts: the *s*-region, which encodes the signal peptide with the *s1*or *s2* allele, and the *m*-region (middle) with the *m1* or *m2* allele[6,12]. The mosaic combination of the *s* and *m* region alleles determines the production of the vacuolating cytotoxin and is associated with the pathogenicity of the bacterium[13]. In general, *vacAs1/m1* and *s1/m2* strains produce high and moderate levels of vacuolating toxin, respectively, whereas the *s2/m2* strains produce little or no toxin[12]. The *vacAs1/m1* genotype is considered to be associated with more severe pathologies[14].

The *iceA* gene (induced by contact with the epithelium) has two main allelic variants, designated *iceA1*and *iceA2*. The *iceA1*allele is up-regulated by the contact of *H. pylori* with gastric epithelial cells and has been associated with peptic ulcer disease. Meanwhile, the *iceA2* allele has been related to asymptomatic gastritis and non-ulcer dyspepsia[7,15].

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The *babA* gene (blood-group antigen-binding adhesin*)* encodes a membrane protein, an adhesion called BabA, which binds to the Lewisb blood group antigen on the gastric epithelial cells[8,16]. Although three *bab* alleles have been identified (*babA1*, *babA2* and *babB),* only the *babA2* gene product is necessary for the Lewisb binding activity. Thus, *babA2* is responsible for pathogenicity, allowing contact between bacterium and gastric epithelium and facilitating the release of other pathogenicity factors[11].

We hypothesized that the clinical outcomes of *H. pylori* infection were influenced by the distribution of the above-mentioned pathogenic factors; therefore, this study aimed at investigating the role of *cagE* as a pathogenicity biomarker of *H. pylori*-positive patients, compare it to *cagA, vacA, iceA* and *babA2* genes and correlate these findings with endoscopic diagnoses.

METHODS

Patients and clinical samples

In this study were included 144 patients with dyspeptic symptoms submitted to upper gastrointestinal endoscopy between October 2008 and March 2009 in the Integrated Center for Gastroenterology at the Hospital of the Federal University of Rio Grande, Rio Grande do Sul, Brazil. Patients that had recently (within the last 15 days) received antibiotics or non-steroidal anti-inflammatory drugs (NSAIDs) or had been treated for *H. pylori* or gastrointestinal bleeding in the last seven days, were excluded. The presence of *H. pylori* infection in the subjects was determined by histological examination and detection of the *ureA* gene by polymerase chain reaction (PCR).

Endoscopic diagnosis

The endoscopic diagnosis was established in accordance to the Sydney System classification[17].

Histological examination

The biopsy samples of the gastric antrum and body destined for histology were fixed in formalin and stained with Hematoxylin-Eosin (H&E) and Giemsa. Histological classification of gastritis was established according to the Sydney System[18].

Extraction of DNA

After collection, the biopsy samples of the gastric antrum and body were kept in Brain Heart Infusion Broth (Acumedia®, United States of America) with 20% glycerol and stored at -70 °C for further DNA extraction. DNA was extracted of the biopsy samples using DNAzol® Reagent (Invitrogen™, United States of America) and 10µg/µL proteinase K (Promega, United States of America). The samples were separated from the broth and re-suspended in 100µL of proteinase K and 500µL of DNAzol® Reagent. The mixture was incubated at 55°C for 3h and, after this period, 500µL of DNAzol® Reagent was added to it again. After centrifugation at 14,000g for 10min, the supernatant was collected and 500µL cold absolute ethanol was added, followed by centrifugation at 12,000***g*** for 10min, after which the supernatant was discarded. The DNA pellet was washed two times with 800µL of 75% ethanol, air dried and re-suspended in 50µL of 8mM NaOH. The DNA was stored at -20°C until further usage.

Detection of the *ureA* gene

The detection of the *ureA* gene was used to confirm the *H. pylori* infection in all of the patients[19]. PCRwas performed as described by Rota et al[20].

Detection of pathogenicity genes by PCR

The presence of the *cagA* gene was investigated by the amplification of the constant region near the 3′ end of the *cagA*. The PCR was performed as proposed by Rota et al.[20,21]. and the *cagE* gene was investigated according to Sozzi et al.[22]. The presence of the *vacA* and *iceA* alleles in the biopsy samples was investigated using the primers previously described[23,24] and the PCR was conducted as proposed by Benenson et al[25]. For detection of the *babA2* gene, the primers and the PCR conditions applied, were described by Sheu et al.[26].

Statistical analysis

The chi-square test was used for the analysis of categorical data. *P*-values of less than 0.05 of a two-tailed test were considered statistically significant. The analyses were performed using the software Statistica 10.

Ethical considerations

This study was approved by the Research Ethics Committee of the Health Area (FURG process number 23116.003335/2008-43) and carried out in accordance with the ethical standards outlined in the Helsinki Declaration. A written informed consent was obtained from all the patients.

RESULTS

From the 144 patients who underwent endoscopy, 57 (39.6%) presented *H. pylori* under histological examination and PCR, of these 40 were women and 17 were men with an average age of 46.2 years (range, 14-74 years). Based on the endoscopic diagnoses, 45.6% (26/57) of the patients had erosive gastritis, while 54.4% (31/57) had enanthematous gastritis.

The distribution of the *cagA, cagE, vacA, iceA* and *babA2* genes in relation to the endoscopic diagnoses is described in **Table 1**. A statistically significant association was found between the *cagE* gene and the diagnosis of erosive gastritis (p=0.029). However, between the *cagA, vacA*, *iceA* and *babA2* genes and the clinical manifestations, no statistically significant association was observed, although a biological significance was suggested.

The presence of the *cagA* gene was significantly correlated with the *cagE* gene ($p < 0.001$) and only two *cagA*-positive samples did not present the *cagE* gene **(Table 2)**. A statistically significant association was also observed between the presence of *cagA, cagE* and *vacA* genes versus *babA2* ($p<0.05$)

TABLE 1 - Distribution of the *vacA, cagA*, *cagE*, *iceA* and *babA2* genes in gastric biopsy samples from patients with different gastric disorders

	Enanthematous gastritis (n = 31)		Erosive gastritis (n = 26)	
Genotype	n	%	n	%
vacA[a]				
vacAs1/m1 (n = 15)	6	40.0	9	60.0
vacAs1/m2 (n = 10)	4	40.0	6	60.0
vacAs2/m1 (n = 1)	1	100.0	-	
vacAs2/m2 (n = 12)	10	83.3	2	16.7
vacA-negative (n = 19)	10	52.6	9	47.4
cagA[b]				
cagA-positive (n = 26)	11	42.3	15	57.7
cagA-negative (n = 31)	20	64.5	11	35.5
cagE[c]				
cagE-positive (n = 24)	9	37.5	15	62.5
cagE-negative (n = 33)	22	66.7	11	33.3
iceA[d]				
iceA1 (n = 11)	4	36.4	7	63.6
iceA2 (n = 30)	17	56.7	13	43.3
iceA-negative (n = 16)	10	62.5	6	37.5
babA2[e]				
babA2-positive (n = 32)	19	59.4	13	40.6
babA2-negative (n = 25)	12	48.0	13	52.0

[a]p-value of the Chi-square test = 0.136; [b]p-value of the Chi-square test = 0.094; [c]p-value of the Chi-square test = 0.029; [d]p-value of the Chi-square test = 0.381; [e]p-value of the Chi-square test = 0.392.

(Table 3). We evaluated the distribution of genes in all patients. The combination *cagA*/*cagE* was detected in 62.5% (15/24) and 37.5% (9/24) of patients with erosive and enanthematous gastritis, respectively. The biomarkers *cagA*/*cagE*/*babA2*/*vacAs1m1*/*iceA1*combined was present in 15.4% (4/26) of patients with erosive gastritis. In patients with enanthematous gastritis, the combination *babA2*/*vacAs2m2*/*iceA2* was detected in 22.6% (7/31). Among patients *H. pylori*-positive, 28.1% (16/57) did not show any of the biomarkers studied here.

DISCUSSION

Helicobacter pylori infection has been related to severe gastroduodenal diseases. There is an increasing evidence that the presence of *H. pylori* genes and their different genotypic combinations are related to development of gastric diseases[11].-

The *cagA* gene was detected in 57.7% (15/26) of gastric biopsy samples from patients with erosive gastritis. This gene has often been associated with the apoptosis of T helper type 1 (Th1) cells, increased IL-8 production, increased inflammation in the gastric mucosa and a higher risk for developing peptic ulcers or gastric cancers[27].

TABLE 2 - Relationship between *cagA* and *cagE* genes in gastric biopsy samples

Genotype	*cagE* – positive (n = 24)	*cagE* – negative (n = 33)
cagA – positive (n = 26)	92.3% (24/26)	7.7% (2/26)
cagA – negative (n = 31)	0% (0/31)	100% (31/31)

p value of the Chi-square test < 0.001 .

TABLE 3 - Relationship between the *cagA*, *cagE* and *vacA* genes versus *babA2* in gastric biopsy samples

Genotype	*babA2* – positive (n = 32)	*babA2* – negative (n = 25)
cagA[a]		
cagA – positive (n = 26)	80.8% (21/26)	19.2% (5/26)
cagA – negative (n = 31)	35.5% (11/31)	64.5% (20/31)
cagE[b]		
cagE – positive (n = 24)	79.2% (19/24)	20.8% (5/24)
cagE – negative (n = 33)	39.4% (13/33)	60.6% (20/33)
vacA[c]		
vacAs1/m1 (n = 15)	73.3% (11/15)	26.7% (4/15)
vacAs1/m2 (n = 10)	90.0% (9/10)	10.0% (1/10)
vacAs2/m1 (n = 1)	100.0% (1/1)	-
vacAs2/m2 (n = 12)	83.3% (10/12)	16.7% (2/12)
vacA-negative (n = 19)	5.3% (1/19)	94.7% (18/19)

[a]p-value of the Chi-square test < 0.001; [b]p-value of the Chi-square test = 0.003; [c]p-value of the Chi-square test < 0.001.

On the other hand, the *cagE* gene was identified in 62.5% (15/24) of gastric biopsy samples from patients with erosive gastritis. This study found a statistically significant association between *cagE* and erosive gastritis, a more severe mucosal injury. This may be due to the fact that this gene is directly connected with an increase in the production of IL-8 in the gastric cells and with the intensity of epithelial damage[28].

When evaluating the effect of the combination of genes with the type of gastritis, the presence of the *cagA*/*cagE* combination in patients with erosive gastritis was 62.5% (15/24). In patients with enanthematous gastritis, this combination was detected in 37.5% (9/24). The relation between the variables was statistically significant (p=0.047). These results permit to infer that *cagE* is an important marker of pathogenicity alone or combined with *cagA*.

Concerning the *vacA* gene, the combination *s1/m1* was mostly detected in gastric biopsy samples derived from patients with an endoscopic diagnosis of erosive gastritis. However, the combination *s2/m2* of the *vacA* gene was frequently observed in samples from patients with enanthematous gastritis. In general, *vacAs1/m1*strains have been linked with higher degrees of inflammation and cell infiltration when compared to *vacAs2/m2*strains[29,30]. Furthermore, *vacAs1/m1* strains produce large

amounts of vacuolating toxin and induce a higher vacuolating activity in gastric epithelial cells than *vacAs2/m2* strains[12,31].

Regarding the *iceA* gene, the *iceA1* allele was more commonly found in samples from patients with erosive gastritis (63.6%), whereas the *iceA2* allele was more commonly identified in specimens from patients with enanthematous gastritis (56.7%), but no statistically significant association was observed. A previous study demonstrated that *iceA1* expression was significantly related to the host mucosal response, which led to the hypothesis that the levels of transcription within the host environment may contribute to disease development. In contrast, *iceA2* expression may be more influenced by the gene structure, which has a repeated protein structure but is not homologous with known proteins[7].

A statistically significant association was observed between *cagA, cagE, vacA* genes and *babA2* ($p<0.05$) **(Table 3)**, but other authors did not find any association between these pathogenicity genes in the samples they investigated[23,32,33]. Our data, however, supports the relationship between the genes *cagA, cagE, vacA* and *babA2* that was described in previous reports[34-37].

The association of biomarkers *cagA*/*cagE*/*babA2*/*vacAs1m1*/*iceA1* was detected in 15.4% (4/26) of patients with erosive gastritis, this is such an important evidence, considering that these genotypes are more pathogenic. Similar percentages were found by studies conducted in Colombia and in Brazil. In the latter, only the *iceA* gene was discordant[38,39]. In patients with enanthematous gastritis, the combination of *babA2*/*vacAs2m2*/*iceA2* was detected in 22.6% (7/31). In a previous study in southern Brazil, the *vacAs2m2* and *iceA2* alleles were also related with enanthematous gastritis[40].

According to the results, we concluded that the detection of *H. pylori* is not in itself sufficient to assess the development of gastric mucosal damage, but the presence of pathogenicity genes is able to give such information. Although the small number of samples can be a limitation in this study, these findings highlight the importance of the detection of biomarkers to evaluate the need of treatment for the microorganism eradication, since in some cases the elimination can lead to the development of other pathologies such as gastric esophageal reflux, asthma and obesity[41]. The *cagE* gene can be used as a risk biomarker for gastric lesions contributing to a better assessment of the pathogenic potential of *H. pylori* and for the infection prognosis of the gastric mucosa.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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