Short Communication

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Differences in virulence markers between *Helicobacter pylori* strains from the Brazilian Amazon region

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ABSTRACT

Introduction: This study compares virulence markers of *Helicobacter pylori* isolated from patients in 2 cities in the Brazilian Amazon. **Methods:** The study analyzed 168 patients with chronic gastritis from Belém and 151 from Bragança, State of Pará, Brazil. Levels of bacterial DNA associated with *cagA* and *vacA* alleles were checked by PCR, and hematoxylin-eosin staining was used for histologic diagnosis. **Results:** In Bragança 87% of patients were genotype *s1m1 cagA*-positive (*s1m1 cagA*$^+$), compared with 76% in Belém. In samples from patients in both cities, there was an association between *s1m1 cagA*$^+$ strains and gastric mucosal damage. **Conclusions:** Both cities have a high frequency of *s1m1 cagA*$^+$ strains of *H. pylori*.

Keywords: *Helicobacter pylori.* Vacuolating cytotoxin. Cytotoxin-associated.

Helicobacter pylori is one of the most common pathogens affecting humans, reported to infect approximately 35% to 70% of the world's population. Many individuals infected with *H. pylori* will develop asymptomatic gastritis, but 10% develop peptic ulcer (gastric or duodenal) or gastric cancer. The clinical outcome of the infection depends on a combination of bacterial, host, and environmental factors[1].

Different virulence genes, such as the cytotoxin-associated (*cagA*) gene and the vacuolating cytotoxin (*vacA*) gene, have been described in *H. pylori* infections. Studies conducted in Brazil have shown an association between the s1m1 genotype and *cagA* positivity and the development of gastrointestinal diseases such as peptic ulcers and gastric cancer[2-4]. According to data from the National Cancer Institute (INCA), the frequency of gastric diseases such as peptic ulcers and gastric adenocarcinoma is high in the northern region of Brazil, particularly in the State of Pará[5]. Most cases of gastric cancer have been reported in the northeastern municipalities of the state. However, only a few studies have investigated the occurrence of *H. pylori* in the State of Pará, and these studies have been restricted to the capital, Belém. In Belém, there is a high prevalence of *H. pylori* infection among adult patients with gastric disorders, which ranges from 64% to 74% in patients with gastritis[4,6] and is 82% in patients with gastric ulcer[7].

The objective of the present study was to evaluate differences in presence of virulence markers (*cagA* and *vacA* genes) between *H. pylori* strains isolated from patients with chronic gastritis in 2 cities within the State of Pará.

Patients

Gastric biopsies were collected from 2 groups. There were 168 patients from Belém with chronic gastritis who were seen at the Ofir Loyola Hospital in Belém and 151 patients from Bragança with chronic gastritis who were seen at the Santo Antonio Maria Zaccaria Hospital in Bragança **(Figure 1)**.

During endoscopy, 4 gastric biopsy fragments were collected. Two antral biopsies were analyzed by histological methods, and 2 antral specimens were also analyzed by molecular methods.

Histological evaluation

The biopsies were fixed in 10% buffered formalin solution, processed in alcohols, and embedded in paraffin, and 4 μm thick sequential sections were cut and stained with hematoxylin and eosin. The histopathological findings of chronic inflammation and polymorphonuclear activity were scored on a scale from 0 to 3 using the criteria described in the updated Sydney classification system[1,8], with 0 indicating no inflammation, 1 light inflammation, 2 moderate inflammation, and 3 severe inflammation.

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FIGURE 1 - Geographic localization of Belém and Bragança cities, State of Pará, Brazil.

Deoxyribonucleic acid extraction

Total DNA was extracted from frozen gastric biopsy specimens by the addition of 10µL proteinase K and 300µL lysis buffer (200mM Tris-HCl, 25mM EDTA, 300mM NaCl, 1.2% sodium dodecyl sulfate). The lysate was extracted with an equal volume of phenol-chloroform, precipitated with isopropanol, and washed with 70% ethanol.

PCR amplification and detection of amplified DNA products

Polymerase chain reaction (PCR) amplification for the detection of *H. pylori* DNA in gastric mucosa was performed as described previously[9]. The previously described F1-F and B1-R primers[10] were used for detection of *cagA*, and *vacA* was amplified using the oligonucleotide primers described by Atherton et al.[11], the *vacA* signal region (*vacA s1* or *s2*: primers SS1-F and SS2-F/VA1-R, respectively) and middle region (*vacA m1* or *m2*: primers VA3-F/VA3-R and VA4-F/VA4-R, respectively).

The PCR products were visualized by electrophoresis on 2% agarose gel stained with ethidium bromide and examined under UV illumination.

Statistical analysis

Data were analyzed using Bioestat 5.0 software (available in http://www.mamiraua.org.br/downloads/programas). The log-likelihood ratio G-test with Yates' correction for continuity[12] was used to compare frequencies and to evaluate the association between bacterial genotypes and histological findings.

The G test was used to compare frequencies, and to evaluate the association between bacterial genotypes and histological findings. A level of significance of 5% was adopted.

The epidemiological data of the 2 groups showed what the mean age of patients from Belém, the state capital, was 45 years (range: 18 to 91 years), and the mean age of patients from Bragança was 36 years (range: 18 to 64 years).

Infection with *H. pylori* was more frequent among patients from Bragança. Bacterial DNA was observed in 142 (94%) of the 151 patients in Bragança, while *H. pylori* DNA of was isolated in 130 (77%) of the 168 patients in Belém. Five of the 130 patients from Belém and 12 of the 142 patients from Bragança were co-infected with at least two different *H. pylori* isolates, because the DNA associated with both the *m1* and the *m2* alleles was detected. Thus, the number of isolates analyzed for the prevalence of *cagA* and allelic variants of *vacA* was reduced to 125 in Belém and to 130 in Bragança.

The results of the amplification of the different alleles of the 2 major *H. pylori* virulence factors, *cagA* and *vacA*, are shown in **Figure 2**. All possible combinations of the *vacA* alleles were identified. The most prevalent *vacA* s-region genotype was *s1*, whose frequency ranged from 79% (99/125) in Belém to 95% (124/130) in Bragança. For the *vacA* m-region, genotype *m1* was the most prevalent among Belém strains (80%, 100/125) and among Bragança strains (96%, 125/130). The most frequent combination of *vacA* alleles found in patients from both Belém and Bragança was *s1m1*, with a significant difference between the 2 cities ($G = 20.63$, $p \leq 0.01$).

The *cagA* gene was detected in 95/125 (76%) of Belém patients and in 87% (113/130) of Bragança patients, with no significant differences between the 2 cities.

Analysis of the association between the degree of inflammation and neutrophil activity and the 2 major *H. pylori* virulence factors, *cagA* and *vacA*, indicated a higher degree of inflammation and neutrophil activity in patients infected with *s1m1 cagA*-positive (*s1m1 cagA*+) strains when compared to non-virulent strains (*s1m1 cagA*$^-$, *s1m2 cagA*$^-$, *s2m1 cagA*$^-$, *s2m2 cagA*$^-$) **(Table 1)**.

The *H. pylori* genome is genetically diverse and different virulence genes contribute to the risk and severity of disease outcome. Several studies have demonstrated geographical differences in the prevalence of *vac*A alleles and *cag*A status among *H. pylori* isolates[2,3,13].

In the present study, a high prevalence of genotype *s1m1 cagA*$^+$ was observed among patients from the 2 cities studied. The prevalence of *s1m1 cagA*$^+$ strains was higher in Bragança than in Belém. However, the incidence of gastric cancer is higher in Belém. This finding might be explained by the fact that reporting of cases is performed by the cancer referral hospital in Belém, and does not consider the origin of the patient as demonstrated by the records of the Brazilian Ministry of Health, which also suggests the underreporting of diagnosed gastric cancer cases in Bragança.

Regardless of potential underreporting of cases in Bragança, mortality due to gastric cancer has been increasing since 2005, from 2/100,000 inhabitants to 6/100,000 inhabitants in 2007 and 15/100,000 inhabitants by 2010. In contrast, these rates remained constant in Belém, with a decline from 187/100,00 inhabitants in 2007 to 166/100,000 inhabitants in 2008[5].

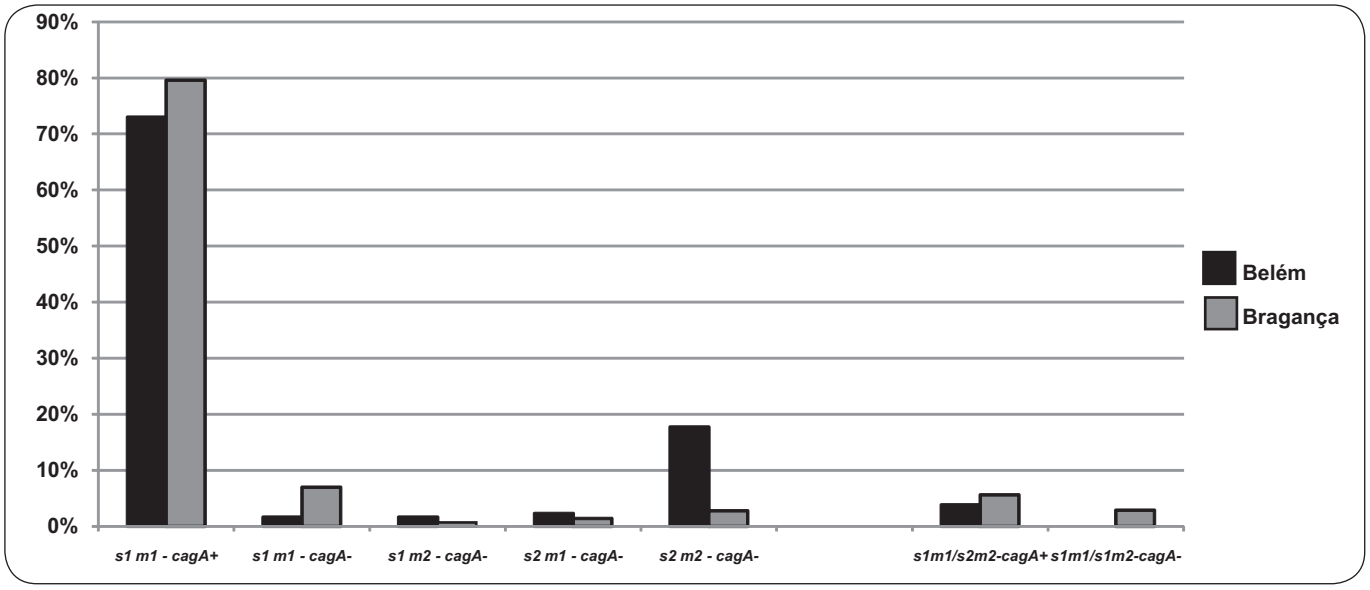

FIGURE 2 - Prevalence of the cytotoxin-associated (*cagA*) gene and of the different allele combinations of vacuolating cytotoxin (*vacA*) gene in patients with chronic gastritis.

TABLE 1 - Association between the degree of inflammation and neutrophil activity and virulence factors (*vacA* and *cagA*) in *Helicobacter pylori*.

	Degree of inflammation				Neutrophil activity			
Genotype	1(%)	2–3 (%)	G test	P	1 (%)	2–3 (%)	G test	P
Belém								
s1m1-cagA+	13 (14.0)	82 (86.0)	19.8632	<0.01	20 (21.0)	75 (79.0)	10,4287	<0.01
NV	12 (70.0)	5 (30.0)			11 (64.0)	6 (36.0)		
Bragança								
s1m1-cagA+	2 (2.0)	111 (98.0)	37.9049	<0.01	4 (3.0)	109 (97.0)	37,7517	<0.01
NV	11 (65.0)	6 (35.0)			12 (70.0)	5 (30.0)		

NV: non-virulent strain (*s1m1 cagA*$^{-}$, *s1m2 cagA*$^{-}$, *s2m1 cagA*$^{-}$, *s2m2 cagA*$^{-}$).

Studies conducted in Brazil have shown that *vacA s1m1* and *cagA*$^{+}$ genotypes increase the risk of gastric cancer and peptic ulcers[2-4]. In the present study, comparison of bacterial genotypes and histopathological findings showed that patients from the 2 cities who carried *s1m1 cagA*$^{+}$ strains had a higher degree of inflammation and neutrophil activity in the gastric mucosa.

Helicobacter pylori is a well-established risk factor, but not a sufficient cause for the development of stomach cancer[14]. In this respect, numerous epidemiological studies have indicated diet to be an important exogenous risk factor[14,15]. There is marked diversity in dietary habits in the Amazon region as a whole due to differences in environmental conditions. Whereas the diet of the rural population consists of fruits, game, and fish, complemented by cassava flour, the source of protein for the population living in the state capital is often dried fish and beef jerky, along with a high intake of canned products[14,15]. Other lifestyle-related factors such as smoking, alcohol consumption, and stress, which have been associated with gastric carcinogenesis, are also more frequent in urban areas[15].

Therefore, in addition to *H. pylori* infection, other factors probably contribute to the elevated incidence of gastric cancer in the cities of the State of Pará.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Kusters JG, Van Vliet AHM, Kuipers EJ. Pathogenesis of *Helicobacter pylori* Infection. Clin Microbiol Rev 2006; 19:449-490.
2. Ashour AAR, Magalhães PP, Mendes EN, Collares GB, Gusmão VR, Queiroz DMM, et al. Distribution of *vacA genotypes* in *Helicobacter pylori* strains isolated from Brazilian adult patients with gastritis, duodenal ulcer or gastric carcinoma. FEMS Immunol Med Microbiol 2002; 33:173-178.
3. Brito CAA, Silva LMB, Jucá N, Leal NC, Souza W, Queiroz DMM, et al. Prevalence of *cagA* and *vacA* genes in isolates from patients with *Helicobacter pylori*-associated gastroduodenal diseases in Recife, Pernambuco, Brazil. Mem Inst Oswaldo Cruz 2003; 98:817-821.

4. Martins LC, Corvelo TC, Demachki S, Araujo MT, Assumpção MB, Vilar SC, et al. Clinical and pathological importance of vacA allele heterogeneity and cagA status in peptic ulcer disease in patients from North Brazil. Mem Inst Oswaldo Cruz 2005; 100: 875-881.
5. Ministério da Saúde. Instituto Nacional do Câncer (INCA). Estimativas da incidência por Câncer no Brasil para o ano 2010. Rio de Janeiro: INCA; 2009/2010.
6. Aguiar DCF, Corvelo TC, Araújo M, Cruz EM, Daibes S, Assumpção MB. Expressão dos antígenos ABH e Lewis na gastrite crônica e alterações pré-neoplásticas da mucosa gástrica. Arq Gastroenterol 2002; 39: 222-232.
7. Martins LC, Corvelo TCO, Oti HT, Barile KAS. Soroprevalência de anticorpos contra o antígeno cagA do *Helicobacter pylori* em pacientes com úlcera gástrica na região Norte do Brasil. Rev Soc Bras Med Trop 2002; 35:307-310.
8. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The Updated Sidney System, International Workshop on the Histopathology of Gastritis, Houston 1994. American J Surg and Pathol 1996; 20:1161-1181.
9. Hammar M, Tyszkiewicz T, Wadstrom T, O'Toole PW. Rapid detection of *Helicobacter pylori* in gastric biopsy material by polymerase chain reaction. J Clin Microbiol 1992; 30:54-58.
10. Tummuru MK, Cover TL, Blaser MJ. Cloning and expression of a high molecular mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. Infect Immun 1993; 61:1799-1809.
11. Atherton JC, Cao P, Peek Jr RM, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem 1995; 270:17771-17777.
12. Sokal RR, Rohlf FJ. Biometry: the principles and practice of statistics in biological research. 4th edition. New York: Freeman & Company, 2011.
13. Van Doorn LJ, Figueiredo C, Megraud F, Pena S, Midolo P, Queiroz DM, et al. Geographic distribution of *vacA* allelic types of *Helicobacter pylori*. Gastroenterol 1999; 116:823-830.
14. Resende ALS, Mattos IE, Koifman S. Mortalidade por câncer gástrico no Estado do Pará, 1980-1997. Arq Gastroenterol 2006; 43:247-252.
15. Palli D. Epidemiology of gastric cancer: an evaluation of available evidence. J Gastroenterol 2000; 35:84-89.