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


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 infection among adult patients with gastric disorders, which ranges from 64% to 74% in patients with gastritis and is 82% in patients with gastric ulcer.

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, with 0 indicating no inflammation, 1 light inflammation, 2 moderate inflammation, and 3 severe inflammation.
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 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 *ml* 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 ≤ 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 *vacA* alleles and *cagA* status among *H. pylori* isolates.

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 187/100,000 inhabitants in 2007 to 166/100,000 inhabitants in 2008.

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**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. The previously described F1-F and B1-R primers were used for detection of *cagA*, and *vacA* was amplified using the oligonucleotide primers described by Atherton et al., 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](http://www.mamiraua.org.br/downloads/programas)). The log-likelihood ratio G-test with Yates’ correction for continuity 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.
Studies conducted in Brazil have shown that \textit{vacA s1m1} and \textit{cagA} genotypes increase the risk of gastric cancer and peptic ulcers\cite{2,3}. In the present study, comparison of bacterial genotypes and histopathological findings showed that patients from the 2 cities who carried \textit{s1m1 cagA} \textsuperscript{+} strains had a higher degree of inflammation and neutrophil activity in the gastric mucosa.

\textit{Helicobacter pylori} is a well-established risk factor, but not a sufficient cause for the development of stomach cancer\cite{14}. In this respect, numerous epidemiological studies have indicated diet to be an important exogenous risk factor\cite{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\cite{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\cite{15}.

Therefore, in addition to \textit{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**

