# *Helicobacter pylori vacA* and *cagA* genotypes in patients from northeastern Brazil with upper gastrointestinal diseases

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Helicobacter pylori *causes chronic gastric inflammation and significantly increases the risk of duodenal and gastric ulcer disease and distal gastric carcinoma. In this study, we evaluated the* Helicobacter pylori vacA *and* cagA *genotypes in patients from a Brazilian region where there is a high prevalence of gastric cancer. Polymerase chain reaction (PCR) was used to investigate* vacA *mosaicism and* cagA *status in the gastric mucosa of 134* H. pylori*-positive patients, including 76 with gastritis: 28 with peptic ulcer disease and 30 with gastric cancer. The s1m1 variant was the predominant* vacA *genotype observed, whereas the s1 allele was more frequently observed in patients with more severe diseases associated with* H. pylori *infection [p = 0.03, odds ratio (OR) = 5.72, 95% confidence interval (CI) = 1.15-38.60]. Furthermore, all of the s1 alleles were s1b. Mixed* vacA *m1/m2 strains were found more frequently in patients with gastric cancer and a* cagA*-positive status was significantly associated with gastric cancer (p = 0.016, OR = 10.36, 95% CI = 1.35-217.31). Patients with gastric cancer (21/21, 100%, p = 0.006) or peptic ulcers (20/21, 95%, p = 0.02) were more frequently colonised by more virulent* H. pylori *strains compared to gastritis patients (41/61, 67.2%). In conclusion, in the northeastern of Brazil, which is one of the regions with the highest prevalence of gastric cancer in the country, infection with the most virulent* H. pylori *strains, carrying the* cagA *gene and s1m1* vacA *alleles, predominates and is correlated with more severe* H. pylori*-associated diseases.*

Key words: *H. pylori vacA - cagA* gastric diseases

*Helicobacter pylori* is a gastric pathogen that causes chronic gastric inflammation and significantly increases the risk of developing duodenal and gastric ulcer disease, distal gastric carcinoma and gastric lymphoma (Suerbaum & Michetti 2002). A high level of phenotypic and genotypic diversity is observed among *H. pylori* strains, especially with respect to virulence markers that confer increased pathogenicity to the bacterium (Suerbaum & Michetti 2002). In the present study, we used molecular methods to evaluate the prevalence of *H. pylori* virulence genes in the gastric mucosa of patients with gastritis, peptic ulcers and gastric carcinoma in Fortaleza, state of Ceará, Brazil, a region with a high prevalence of gastric cancer and *H. pylori* infections (Motta et al. 2008). We included 134 non-consecutive patients in the study group from Walter Cantídeo University Hospital, who were infected with *H. pylori* strains (30 with gastric cancer, 28 with peptic ulcer and 76 with gastritis) and were undergoing upper gastrointestinal endoscopy or gastric surgery to remove gastric carcinoma. The study was approved by the Ethical Committee of the institution and informed consent was obtained from all included patients.

During upper gastrointestinal endoscopy, five biopsy specimens were obtained for histological evaluation according to the Houston-Updated Sydney System for the classification of gastritis (Dixon et al. 1996). Additionally, two fragments were collected from the antral mucosa for rapid urease testing and for DNA analysis to detect *H. pylori* virulence genes. Gastric mucosa fragments were obtained from tumours from gastric cancer patients, as was normal tissue (collected 5 cm away from the tumour).

DNA was extracted from the antral gastric specimens using the QIAamp (QIAgen®, Hilden, Germany) kit according to the manufacturer's recommendations. The presence of the *H. pylori*-specific *ureA* gene was evaluated according to a previously reported methodology (Queiroz et al. 2005).

Polymerase chain reaction amplification of the *vacA* signal sequence and midregion was performed as described previously (Ashour et al. 2002). The *H. pylori* strains were initially classified as type s1 or s2 and m1 or m2, and the s1 genotype was further characterised into s1a, s1b or s1c variants (Ashour et al. 2002). The *cagA* gene was amplified as described previously (Queiroz et al. 2005). Negative and positive controls were included in all reactions. To evaluate associations between the disease and *vacA* mosaicism we excluded mixed infections from the analysis. The groups were compared using the two-tailed chi-square or Fisher's exact test. A value of $p \leq 0.05$ was considered significant.

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The *H. pylori*-specific *ureA* gene was detected in the gastric mucosa of all patients. Demographic data on the patients and their *vacA* mosaicism and *cagA* status are shown in Table I. Excluding s1/s2 mixed infections, which were observed more frequently (p = 0.054) in peptic ulcer (25%) than gastritis (9.2%) or cancer (13.4%) patients, most of the strains carried the s1 *vacA* genotype was observed in 100 (74.6%) cases. Among them, 83 strains were further characterised into s1 variants and all of which were s1b. The s1 genotype was observed in 72.4%, 71.4% and 83.3% of the strains obtained from patients with gastritis, gastroduodenal ulcer and gastric cancer, respectively, and tended to be more frequent in gastric cancer patients (p = 0.06). When the strains from patients with peptic ulcer and gastric cancer were evaluated together, the difference became significant (p = 0.029, odds ratio (OR) = 5.72, 95% confidence interval (CI) = 1.15-38.60). The most virulent *vacA* s1 allele was highly predominant among the *H. pylori* strains from the northeastern region of Brazil, even in the group of patients without severe diseases associated with *H. pylori* infection. Similar findings have been reported in other western countries (van Doorn et al. 1999), but not in southeastern Brazil (Ashour et al. 2002). It has been demonstrated that distinct *H. pylori vacA* s and m alleles show a specific geographical distribution (van Doorn et al. 1999). On the Iberian Peninsula and in Latin America, the most frequent *vacA* s1 allele is *vacA* s1b (van Doorn et al. 1999, Ashour et al. 2002). Only the *vacA* s1b allele was identified in this study, which is in agreement with the *vacA* profile of the strains described in southeastern Brazil (van Doorn et al. 1999, Ashour et al. 2002).

The *H. pylori* m1 allele was detected in 74 patients, while the m2 allele was detected in 41 patients. Eight patients expressed both the m1 and m2 alleles, without any difference among the groups (p ≥ 0.27). This distribution is similar to that found on the Iberian Peninsula and in other countries in Latin America (van Doorn et al. 1999). Mixed *vacA* m1/m2 infections were more frequently observed in gastric cancer patients than gastritis patients (p = 0.007, OR = 15.23, 95% CI = 1.57-364.10), which was also in agreement with other studies (Figueiredo

TABLE I

Patient characteristics and distribution of *Helicobacter pylori vacA* and *cagA* genotypes according to the studied diseases

| | Gastritis (n = 76) n (%) | Peptic ulcer (n = 28) n (%) | Gastric cancer (n = 30) n (%) |
|---|---|---|---|
| Mean age (standard deviation) | 41.6 (12.6) | 49.7 (40.6) | 46.8 (14.1) |
| Female sex | 50 (65.9) | 15 (53.6) | 13 (43.3) |
| *vacA* s1 alleles | 55 (72.4) | 20 (71.4) | 25 (83.3) |
| *vacA* s2 alleles | 14 (18.4) | 1 (3.6) | 1 (3.3) |
| *vacA* s1/s2 alleles | 7 (9.2) | 7 (25) | 4 (13.4) |
| *vacA* m1 alleles | 39 (51.3) | 19 (67.8) | 16 (53.3) |
| *vacA* m2 alleles | 28 (36.8) | 7 (25) | 6 (20) |
| *vacA* m1/m2 alleles | 1 (1.3) | 2 (7.1) | 5 (16.7) |
| Non detected *vacA* m allele | 8 (10.9) | 0 (0) | 3 (10) |
| *cagA*-positive | 56 (73.7) | 26 (92.9) | 29 (96.7) |
| *cagA*-negative | 20 (26.3) | 2 (7.1) | 1 (3.3) |

TABLE II

Association of *Helicobacter pylori vacA* and *cagA* genotypes according to the studied diseases

| | Gastritis (n) | | Peptic ulcer (n) | | Gastric cancer (n) | |
|---|---|---|---|---|---|---|
| *vacA* genotype | *cagA*+ | *cagA*- | *cagA*+ | *cagA*- | *cagA*+ | *cagA*- |
| s1m1 | 28 | 7 | 14 | 0 | 16 | 0 |
| s1m2 | 13 | 4 | 5 | 1 | 5 | 0 |
| s2m2 | 5 | 4 | 1 | 0 | 0 | 0 |
| Mixed infection | 3 | 1 | 6 | 1 | 5 | 1 |
| Positive (+) - negative (-) | 7 | 1 | 0 | 0 | 3 | 0 |
| Total | 56 | 20 | 26 | 2 | 29 | 1 |

et al. 2001, Ashour et al. 2002). Eleven patients did not exhibit detectable m alleles. The prevalence of mixed infections by different *vacA* genotypes found in this study was similar to that observed in other studies from the Netherlands (van Doorn et al. 1999) and southeastern Brazil (Ashour et al. 2002), but was lower than that observed in Portugal (Figueiredo et al. 2001) and Mexico (Morales-Espinosa et al. 1999). Mixed infections were found more frequently in patients with severe diseases associated with the infection, which may be attributed to microevolution due to intra-host diversification during long-term colonisation (Aras et al. 2003). The s1m1 *vacA* genotype was the most common *vacA* allelic combination observed (Table I), even in the group of patients without the more severe *H. pylori*-associated diseases. This finding was similar to what has been observed in eastern countries (Yamaoka et al. 1999).

The prevalence of *H. pylori cagA*-positive strains varies by geographic region and has been associated with more severe gastroduodenal diseases in some areas (Oliveira et al. 2003). In the present study, a high prevalence of *cagA*-positive *H. pylori* strains was observed (82.8%) (Table I), which was significantly associated with gastric cancer (p = 0.016, OR = 10.36, 95% CI = 1.35-217.31), as previously described for patients from different countries and from the southeastern and northeastern regions of Brazil (Evans et al. 1998, Umit et al. 2008, Lima et al. 2011).

Previous studies have also shown associations between a *cagA*-positive genotype and the presence of peptic ulcers (Evans et al. 1998, Oliveira et al. 2003, Martins et al. 2005, Umit et al. 2008).

In this study, we observed that infection with *cagA*-positive strains was more prevalent in patients with peptic ulcers than patients with gastritis, but this finding did not achieve statistical significance (p = 0.06, OR = 4.64, 95% CI = 0.94-31.09).

Because the *vacA* s1 allele was associated with *cagA*-positive status (p = 0.02, OR = 5.19, 95% CI = 1.16-23.40) and the *vacA* s1m1 genotype tended to be observed among *cagA*-positive strains (p = 0.08) (Table II), we examined whether the *cagA* + *vacA* s1m1 and *cagA* + *vacA* s1m2 strains, which are the most virulent *H. pylori* strains, were associated with severe *H. pylori*-associated disease by analysing only the strains genotyped for all of the investigated genes and excluding mixed infections. Patients with gastric cancer (21/21, 100%, p = 0.006) and peptic ulcers (20/21, 95%, p = 0.02) were more frequently colonised by the most virulent *H. pylori* strains compared to gastritis patients (41/61, 67.2%) (Table II).

In conclusion, in the northeastern region of Brazil, which is the region with the highest prevalence of gastric cancer, infection by the most virulent *H. pylori* strains, carrying the *cagA* gene and the s1m1 *vacA* alleles, predominates and is correlated with more severe *H. pylori*-associated diseases.

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