Association of LEC and tnpA Helicobacter pylori genes with gastric cancer in a Brazilian population

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Abstract

Background: H. pylori seroprevalence in Brazilians varies and is dependent on socioeconomic status, sanitation conditions and ethnicity; furthermore, H. pylori is not always associated with the incidence of gastric cancer, suggesting the role of more virulent strains. The purpose of this study was to analyze the association of more virulent H. pylori strains with gastric cancer.

Methods: DNA was extracted from gastric biopsies of thirty-four cases of gastric cancer (11 intestinal-type, 23 diffuse-type), and thirty-four of patients with endoscopic gastritis. The presence of cagPAI genes (cagA, cagA promoter, cagE, cagM, tnpB, tnpA, cagT and the left end of the cagII (LEC)) and babA were analyzed by PCR.

Results: Comparison of H. pylori isolates from gastric cancer and gastritis patients showed significant associations of tnpA and LEC with gastric cancer (73.5% [OR, 6.66; 95% CI, 2.30-19.25] and 58.8% [OR, 10.71; 95% CI, 3.07-37.28] of cases, respectively). Other cagPAI genes were detected in both groups at similar frequencies.

Conclusions: tnpA and LEC of H. pylori cagPAI were associated with gastric cancer; nonetheless, these results were restricted within this group of patients and further studies are needed to confirm these results in a larger sample and determine their role in gastric carcinogenesis.

Findings

The seroprevalence of H. pylori ranges among Brazilians, and is dependent on age, socioeconomic status, and ethnicity, as well as sanitation conditions. High prevalence has been reported in the city of Fortaleza, with 73.3% positive cases in individuals 11-20 years old, and up to 87% in those over 60 years old [1]. Notably, the incidence rate of gastric cancer is lower (16/100,000) in Fortaleza than in the city of São Paulo (22/100,000) http://www.inca.gov.br/, which has a lower prevalence of H. pylori (65.6%) [2]. Furthermore, H. pylori seroprevalence was higher in African Brazilians compared to Caucasoids [2] and Japanese Brazilians [3], despite the higher mortality for gastric cancer among Japanese Brazilians compared to the indigenous population [4]. These conflicting results suggest among other factors that more virulent H. pylori strains may be involved in gastric cancer outcome.

The cag (cytotoxin-associated gene) pathogenicity island (cagPAI) in H. pylori contains 31 putative genes [5] and encodes a type IV secretion system that delivers CagA into the cytosol, which is phosphorylated and activates phosphatase activity to initiate morphological changes of the cell, providing a potential mechanism by which chronic H. pylori infection may promote the development of gastric cancer [6]. We previously showed that the cagPAI genes cagT, cagM, cagA and LEC (the left end of the cagII) were significantly associated with peptic ulcers [7], and these findings were later confirmed by another group in Brazil [8]. The purpose of this study was to analyze the association of cagPAI genes and the blood group antigen binding adhesin babA of H. pylori with gastric cancer.

Patients were from the indigenous population of the city of São Paulo and were classified according to ethnicity as White, Brown (of White and African-Brazilian descent) and Black, none was Japanese-Brazilian. Among
68 consecutive gastric cancer patients, only 34 (11 intestinal-type, 23 diffuse-type) were H. pylori positive, 20 of which were men and 14 were women; the patients’ mean age was 53.3 ± 12.4 years, and ranged from 24 to 75 years old; the median age was 52.5 years; 25 were White, 7 were Brown and 2 were Black.

Controls were selected among dyspeptic patients without previous history of peptic ulcer and gastric cancer, presenting a recent diagnosis of only gastritis (superficial or erosive) by upper gastrointestinal endoscopy, and positive for H. pylori infection. Of the 34 controls, 23 were women and 11 were men, with a mean age of 50.9 ± 9.8 years and a range from 32 to 70 years old; the median age was 48.5 years; 29 were White, 4 were Brown and 1 was Black.

The criteria for H. pylori positivity were the same for patients and controls, positive urease test and PCR with primers that amplify the species-specific 26-kDa antigen gene, using antrum and corpus biopsies [7,9]. Patients and controls had not taken antimicrobials and acid suppression drugs for at least 30 days before the sample collection. In patients with gastric cancer, fragments were obtained from the normal appearance mucosa rather than the cancerous tissue. All the cases were confirmed by histopathological examination. PCR analysis amplified the PAI genes, babA, and DNA sequence of a 26-kDa species-specific protein antigen present in all strains of H. pylori [7,9]. All the cases were confirmed H. pylori positive by urease test and by PCR for the species-specific antigen. Fisher’s exact probability test was determined using SPSS, and the odds ratio OR [95% CI] were calculated using Microsoft Office Excel 2003. A value of P < 0.05 was considered statistically significant. The [OR] and [95% CI] are depicted in Table 1.

Comparison of the presence of cagPAI genes in H. pylori isolates from patients with gastric cancer and gastritis revealed that only two genes were significantly associated with gastric cancer: tnpA, detected in 73.5% (25/34) of the gastric cancer cases, with an odds ratio of 6.66 [95% CI, 2.30-19.25], and LEC, observed in 58.8% (20/34) of cases, with an odds ratio of 10.71 [95% CI, 3.07-37.28]. The other cagPAI genes analyzed in this study were detected equally in both groups. cagA was more frequently found in the gastric cancer group, though this difference was not significant; however, its presence may still be associated with gastric cancer, due to the odds ratio of 1.77 [CI 95%; 0.61-5.11]. The babA gene was more often found in H. pylori isolates from patients with gastritis (64.7%, 22/34) than in isolates from gastric cancer (41.2%, 14/34; p = 0.052), [OR, 0.38; 95% CI, 0.14-1.01]. The analysis of cagPAI and babA genes among the histological types (diffuse and intestinal) of gastric cancer, gender and the ethnic groups revealed no significant difference, data not shown.

As shown in Table 2, H. pylori isolates from gastric cancer cases were usually positive for both LEC and tnpA (p = 0.017 by Fisher’s exact test). In Japan association of IS605 with other cagPAI genes, cag13 and cagA was previously reported in gastric cancer cases. However, as the role of this finding remains unclear, further study is necessary to determine its involvement in gastric cancer [10].

In this study, we analyzed the prevalence of H. pylori genes in patients from a Brazilian population with gastric cancer or gastritis. Unexpectedly, babA, the blood group antigen binding adhesion targeting human Lewis surface epitopes on gastric epithelial cells associated with duodenal ulcer and gastric adenocarcinoma [11], was frequently deleted in gastric cancer H. pylori isolates (41.2%), compared to gastritis (64.7%); nonetheless, in the previously studied peptic ulcer group, 69.3% of H. pylori isolates also were babA positive [9]. Thus, babA may be a frequent genotype with no particular role in the clinical outcome [12].

We previously showed that the cagPAI genes cagT, cagA, cagM and LEC were associated with peptic ulceration progression [7]; this study revealed that only LEC

### Table 1 Prevalence of cagPAI genes and babA in H. pylori isolates from gastritis and gastric cancer patients.

| Genes   | Controls  | Gastric cancer  | P value | OR  | 95% CI          |
|---------|-----------|-----------------|---------|-----|-----------------|
| cagA    | 8 (23.5%) | 12 (35.3%)      | P = 0.287 | 1.77 | 0.61-5.11      |
| cagE    | 24 (70.6%)| 22 (64.7%)      | P = 0.604 | 0.76 | 0.27-2.11      |
| cagM    | 8 (23.5%) | 6 (17.6%)       | P = 0.549 | 0.69 | 0.21-2.27      |
| cagT    | 16 (47.1%)| 16 (47.1%)      | P = 1.000 | 1.00 | 0.38-25.9      |
| apcA    | 20 (58.8%)| 23 (67.6%)      | P = 0.451 | 1.46 | 0.54-3.94      |
| LEC     | 4 (11.8%) | 20 (58.8%)      | P < 0.0001 | 10.71 | 3.07-37.28     |
| tnpA    | 10 (29.4%)| 25 (73.5%)      | P < 0.0001 | 6.66 | 2.30-19.25     |
| tnpB    | 1 (2.9%)  | 2 (5.9%)        | P = 0.551 | 2.06 | 0.17-23.88     |
| babA    | 22 (64.7%)| 14 (41.2%)      | P = 0.052 | 0.38 | 0.14-1.01      |

P < 0.0001 by Fisher’s exact probability test

### Table 2 Association of tnpA and LEC in H. pylori isolates from patients with gastric cancer.

| Gastric cancer patients (n = 34) | tnpA - | tnpA + | Total |
|----------------------------------|--------|--------|-------|
| LEC -                            | 7      | 7      | 14    |
| LEC +*                           | 2      | 18*    | 20    |
| Total                            | 9      | 25     | 34    |

*p = 0.017 by Fisher’s exact probability test
and \textit{tnpA} were associated with gastric cancer. In South Africa, LEC was frequently deleted in isolates from gastri
titis compared to those from gastric cancer and peptic ulcers [13]. Nevertheless, LEC was not necessary for ei
erther the translocation of CagA that mediates intracel
dular disruption of growth regulation [14], or for the
induction of IL-8 [15], proinflammatory cytokine released upon \textit{H. pylori} infection by gastric epithelial
ells that induced expression and activation of epidermal
growth factor receptor and proliferation [16]. Our find-
ing is consistent with a previous study reporting higher
frequency of \textit{tnpA} in Peruvian gastric cancer strains
than in gastritis strains (9 of 14 versus 15 of 45, respec-
tively; \textit{P} = 0.04) [17].

We did not observe any significant association of \textit{cagA}
with cases of gastric cancer or gastritis, and this is con-
sistent with previous reports in South Africa [13] and
Germany [18], which failed to detect an association with
gastric cancer.

Our study of a population in Brazil indicates an asso-
ciation with LEC and \textit{tnpA} and gastric cancer; nonethe-
less, further studies are needed to confirm these results
in a larger sample, as no specific role for these genes in
gastric carcinogenesis has yet been identified.

**Abbreviations**

cagPAI: cytotoxin-associated gene pathogenicity island; LEC: left end of cag
region

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**Authors’ contributions**

RM participated in the conception, design, analysis and interpretation of
data, statistical analysis, drafted the manuscript and revised final version;
MSM carried out the molecular genetic studies; SBM and CLH obtained
data, statistical analysis, drafted the manuscript and revised final version;
RM participated in the conception, design, analysis and interpretation of
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**Competing interests**

The authors declare that they have no competing interests.

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