Role of Corpus Gastritis and cagA-Positive Helicobacter pylori Infection in Reflux Esophagitis

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Considering that the role of Helicobacter pylori infection in gastroesophageal reflux and reflux esophagitis (GERD) is still controversial and that the role of virulence markers of the bacterium has not been evaluated in most studies of GERD, we investigated the association among H. pylori infection with cagA-positive and -negative strains, corpus gastritis, and GERD in a large group of patients by controlling for confounding factors. We studied prospectively 281 consecutive adult patients: 93 with GERD and 188 controls. H. pylori infection status was diagnosed by culture, by the preformed urease test, with a carbolchufsin-stained smear, and by histology. The cagA status was determined by PCR of H. pylori isolates and gastric biopsy specimens. H. pylori infection was diagnosed in 191 (68.0%) of 281 patients. Among the 93 patients with GERD, 84 presented with mild or moderate esophagitis and 9 presented with severe esophagitis. In the multivariate analysis, the age of the patients and the degree of oxyntic gastritis were associated with GERD. Among the strains isolated from patients with GERD and from the control group, 24.4 and 66.9%, respectively, were positive for cagA (P < 0.001). Compared to infection with cagA-negative strains, infection with cagA-positive H. pylori strains was associated with a more intense gastritis in the corpus (P = 0.001); cagA status (odds ratio [OR] = 0.16, 95% confidence interval [CI] = 0.07 to 0.40), gastritis of the corpus (OR = 0.69, 95% CI = 0.48 to 0.99), and age (OR = 1.04, 95% CI = 1.01 to 1.07) were associated with GERD. In conclusion, the study provides evidence supporting the independent protective roles of cagA-positive H. pylori strains and the degree of corpus gastritis against GERD.

Helicobacter pylori infection is probably one of the most common chronic bacterial infections worldwide. The infection is predominantly acquired in childhood, and in most subjects it runs its course without complications. Nevertheless, a small percentage of infected individuals develop peptic ulcer disease (17), distal gastric carcinoma (19), or mucosa-associated lymphoid tissue lymphoma (28).

Chronic gastroesophageal reflux and reflux esophagitis (GERD) are considered peptic diseases. In recent decades, while the prevalence of H. pylori infection has been declining, the incidence of GERD has been increasing in developed countries (7). Case-control studies (9, 11, 14) have shown that the eradication of H. pylori from patients with duodenal ulcers may provoke GERD. This finding, however, has not been confirmed by other investigators (23, 24). Conflicting data have been also observed in studies evaluating the association between GERD and H. pylori infection. In some of the studies, the rates of H. pylori infection were lower in patients with GERD than in control populations (13, 27, 29), whereas in other studies the prevalence of H. pylori infection was similar (18, 23) or even higher (15) in patients with GERD. However, the small number of subjects, inconsistent methods of determination of H. pylori infection status, or the absence of controls for confounding factors such as age and gender weaken the results of most of these studies and reinforce the need for additional, more controlled evaluations of this subject. Furthermore, the discrepant results may be due to differences among populations as well as overlaps between groups of H. pylori-positive patients colonized by strains lacking and not lacking virulence markers.

Among the putative bacterial virulence factors, the cagA gene, which has been shown to encode a highly immunogenic protein (CagA), is a marker of the cag pathogenicity island (PAI) (2). Patients colonized by strains containing the cag PAI present with more severe gastric inflammation (1, 22), which may explain the lower levels of acid output and the lower levels of protection against GERD. In fact, studies associating the virulence factors of the bacterium with protection against more severe or complicated lesions of GERD such as distal esophageal carcinoma and Barrett’s esophagus have been published (3, 10, 25). It must be emphasized that the role of cagA status in offering protection against less severe forms of GERD was not evaluated or demonstrated in the previous studies. Recently, Warburton-Timms et al. (23) have shown in a well-controlled trial a negative association between cagA status and GERD without complications, but they did not investigate the role of corpus gastritis.

Therefore, considering that there is no consensus regarding the role of H. pylori infection in protecting against GERD and that the role of virulence markers of the bacterium have not been evaluated in most studies, our aim was to investigate the association among H. pylori infection, the presence of cagA-
positive and -negative strains, the degree of corpus gastritis, and GERD (especially without complications) in a large group of patients by controlling for confounding factors.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. All patients provided informed consent to participate in this study.

From 1997 to 2000 we studied prospectively 281 consecutive adult patients (117 men and 164 women; mean age, 45.0 ± 12.2 years; age range, 18 to 88 years) who were selected from among those who came to the Gastroesophagoduodenoscopy Service of Celso Affonso de Oliveira or at the Gastrointestinal Service of the University Hospital—UFMG to clarify the origins of symptoms referable to the upper gastrointestinal tract. All patients with peptic ulcer, esophageal carcinoma, gastric carcinoma, esophageal disorders, complications such as gastric perforation or hemorrhage, anatomic obstacles preventing endoscopy, or esophageal varices and patients who had already undergone gastric surgery were excluded from the study. None of the patients had received antimicrobial drugs, H2-receptor antagonists, acid pump inhibitors, or nonsteroidal anti-inflammatory drugs for at least 30 days before the study.

The GERD group consisted of patients with endoscopically proven esophagitis with or without hiatal hernia. Patients from the control group did not have hiatal hernia, symptoms of reflux, esophagitis at endoscopy, or abnormalities upon histologic examination of the esophageal mucosa.

The patients were considered H. pylori positive if the culture of the biopsy specimen was positive or if they were positive by two of the following tests: the preformed urease test, carbolfuchsin-stained smear examination, or examination of a histologic section. The patients were considered H. pylori negative if the results of all tests were negative.

Endoscopy. At endoscopy, biopsy specimens were obtained from the antral and oxyntic gastric mucosa for microbiologic, histologic, and molecular analyses and from the esophageal mucosa for histologic study. Esophagitis was classified as mild (presence of enanthema, edema, or friability), moderate (presence of linear, multiple, or confluent erosions), or severe or complicated (presence of ulcers, strictures, or Barrett’s metaplasia).

Microbiologic study. Tissue samples for culture were maintained in sodium thiglycollate broth (Difco, Detroit, Mich.) at 4°C for a maximum of 1 h, ground separately in a tissue homogenizer (Kontes, Vineland, N.J.), and plated onto petri dishes containing fresh Belo Horizonte medium (21). The plates were incubated at 37°C in a microaerophilic atmosphere obtained by use of a gas generation system (Anaerocult C; Merck, Darmstadt, Germany). The plates were evaluated after 3, 6, 9, and 12 days of incubation. Strains with macroscopic and microscopic morphologies typical of H. pylori, rapidly positive by a urease test, positive by oxidase and catalase reactions, and found to contain ureA were identified as H. pylori. A pool of strains of the microorganism(s) from each patient was maintained at ~80°C in brucella broth supplemented with 30% glycerol.

One antral fragment was placed in a tube containing Christensen’s 2% urea agar, and the tube was examined within 24 h of incubation at 37°C for urea hydrolysis.

One antral biopsy specimen was immediately rubbed on a glass slide and subsequently heat fixed and stained with 40% carbolfuchsin for detection of spiral-shaped bacteria.

PCR analysis. All H. pylori strains, as well as gastric fragments from H. pylori-positive patients from whom the microorganism was not isolated, were evaluated for the presence of cagA and ureC. Two cagA-positive strains (strains ATCC 49503 and NCTC 11637) and one cagA-negative strain (strain Tx 30A) were used as positive and negative controls, respectively, for cagA detection. The same H. pylori strains and two human isolates (Proteus mirabilis and Escherichia coli) were used as positive and negative controls, respectively, in the reaction for ureC detection. A reagent-negative control reaction, in which DNA samples were replaced with distilled water, was done with each batch of amplification to exclude contamination.

The H. pylori strains were thawed, plated onto brain heart infusion agar plates supplemented with 10% sheep blood, and incubated at 37°C under microaerophilic conditions for 3 days. Abundant bacterial growth on a petri dish (diameter, 50 mm) was transferred with a sterile swab to a tube containing 1 ml of phosphate-buffered saline (PBS; pH 7.4).

Tissue or bacterial DNA was suspended in a tube containing 1 ml of PBS; the tube was centrifuged twice at 13,000 × g for 10 min each time; and the contents of the tube were suspended in a buffer containing 8% sucrose, 50 mM Tris HCl (pH 8.0), 50 mM EDTA, and 0.1% Triton X-100. Lysozyme from chicken egg white (Boehringer Mannheim, Indianapolis, Ind.) was added to a final concentration of 3 mg/ml, and the solution was incubated for 12 min at 37°C. Sodium dodecyl sulfate and RNase from bovine pancreas (Boehringer Mannheim, Mannheim, Germany) were added to final concentrations of 0.8 and 0.5 mg/ml, respectively, and the samples were incubated overnight at 37°C in a 5% (wt/vol) cetlytrimethylammonium bromide (CTAB; Sigma, St. Louis, Mo.)–0.7 M NaCl solution (1:10) was added, and the solution was gently mixed and incubated at 55°C for 10 min. The DNA was extracted with equal volumes of phenol and chloroform (1:1) and was precipitated overnight at −20°C in the presence of 0.3 M sodium acetate and 3.1 volumes of absolute ethanol. The DNA precipitates were then pelleted by centrifugation at 16,000 × g for 30 min and allowed to air dry. The pellets were suspended in sterile distilled water, and the DNA was quantified by measuring the optical density at 260 nm.

Genomic DNA was amplified by PCR for the detection of cagA by using two sets of synthetic oligonucleotides primers, as described elsewhere by Kelly et al. (12) and Peek et al. (20). The amplified PCR products were resolved in 1% agarose gels containing Tris-borate-EDTA by using a 100-bp molecular size marker (Gibco BRL, Gaithersburg, Md.). The agarose gels were stained with ethidium bromide and viewed under short-wavelength UV light. The strains were considered cagA positive when at least one of the reactions was positive.

Histologic study. Biopsy samples of the gastric and esophageal mucosa obtained by endoscopy were fixed in 10% formalin and embedded in paraffin, and 5-μm-thick histologic sections were stained with hematoxylin-eosin for histologic analysis. The samples of the gastric mucosa were also stained with carbolfuchsin for H. pylori detection. The slides were examined by two independent pathologists who were not aware of their origins.

The corpus mucosa were analyzed for mononuclear and polymorphonuclear cells, which were scored as follows according to the revised Sydney system (6): none (score, 0), mild (score, 1), moderate (score, 2), or marked (score, 3). The numerical scores were derived by summing the single scores for mononuclear and polymorphonuclear cell infiltration (scores from 0 to 6).

Histologic alterations of the esophageal mucosa such as neutrophil and eosinophil infiltration, increased epithelial thickness, elongation of papillae, erosion, or ulceration were indicative of GERD.

Statistical analysis. Data were analyzed with the SPSS statistical software package (version 10.0; SPSS Inc., Chicago, Ill.). Differences in the sensitivity of culture between the groups were evaluated by the two-tailed Fisher exact test.

Variables such as gender (male or female), mean age, H. pylori infection status (negative or positive), cagA status (negative or positive), inflammatory intensity, and the antral and oxyntic gastritis activities (scored as defined above) were evaluated. The association of each variable with GERD (the dependent variable) was tested by univariate analysis. All variables with P values of 0.25 or less were included in the full model of logistic regression, and variables with P values <0.05 were retained in the model. The odds ratio (OR) and the 95% confidence interval (CI) were used as estimates of the risk.

All patients were included in the first analysis, irrespective of their H. pylori infection status. In a second analysis, in order to better evaluate the role of the infection with a cagA-positive strain, only H. pylori-positive patients were studied.

RESULTS

Of the 281 patients, 191 (68.0%; 75 men and 116 women; mean age, 44.8 ± 15.4 years; age range, 18 to 88 years) were H. pylori positive and 90 (32.2%; 42 men and 48 women; mean age, 45.3 ± 14.6 years; age range, 18 to 80 years) were H. pylori negative. Among the H. pylori-positive patients, the culture result was positive for 132 (92.9%) individuals in the control group and 45 (91.8%) individuals with GERD (P = 0.75).

Ninety-three patients fulfilled the criteria for GERD (Table 1). Among these patients, 84 (90.3%) presented with mild (n = 59) or moderate (n = 25) esophagitis and 9 (9.7%) presented with severe esophagitis. Among the individuals in the last group, Barrett’s esophagus was seen in four patients.

In the univariate analysis, GERD was associated with H. py-
H. pylori infection is characterized by abnormal exposure of the esophageal mucosa to the acid gastric content. In developed countries, while the rates of GERD and carcinoma of the distal esophagus and proximal stomach have risen progressively, the rates of duodenal ulcer and distal gastric adenocarcinoma have decreased in parallel with the decrease in the rates of H. pylori infection (7).

**DISCUSSION**

Gastroesophageal reflux disease is a common disorder characterized by abnormal exposure of the esophageal mucosa to the acid gastric content. In developed countries, while the rates of GERD and carcinoma of the distal esophagus and proximal stomach have risen progressively, the rates of duodenal ulcer and distal gastric adenocarcinoma have decreased in parallel with the decrease in the rates of H. pylori infection (7).

**TABLE 1. Relationship between GERD and variables related to the patients studied**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>With GERD</th>
<th>Without GERD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of men: no. of women</td>
<td>46:47</td>
<td>71:117</td>
<td>0.07</td>
</tr>
<tr>
<td>Mean ± SD age (yr)</td>
<td>49.6 ± 14.9</td>
<td>42.7 ± 14.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age range (yr)</td>
<td>19–88</td>
<td>18–80</td>
<td></td>
</tr>
<tr>
<td>No. (%) of patients H. pylori positive</td>
<td>49 (52.7%)</td>
<td>142 (75.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (range) corpus gastritis score</td>
<td>1.0 (0–5)</td>
<td>2.0 (0–6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Univariate analysis.

Recently, several studies have evaluated the association between H. pylori infection and GERD. Some of them (13, 25, 27, 29) observed a protective effect of H. pylori infection against GERD, whereas this association was not seen in others (17, 26). These contradictory results could be due to bias, inconsistent tests for the diagnosis of H. pylori infection, or confounding factors. Points that strengthen the results of this study include the prospective study design, the large series of patients and controls, the accurate selection of controls, the adjustment for confounding factors, and the more accurate diagnosis of H. pylori infection. Since biopsy specimens may be false negative due to the patchy distribution of H. pylori in the stomach, we used four tests to improve the accuracy of the diagnosis of infection. Also, as has been recommended, in this study a result was considered truly positive only when the culture result was positive or the results of at least two other tests were positive, and a result was considered truly negative when the results of all tests were negative (16).

In the present study, although the prevalence of infection was lower in patients with GERD than in those without GERD, after adjustment for confounding factors, the disease remained associated only with age, being slightly more frequent in older subjects and in those with a less intense oxyntic gastritis. However, since in the univariate analysis H. pylori infection status and the degree of oxyntic gastritis were both negatively associated with GERD, the lack of association between H. pylori infection and GERD in the multivariate analysis may be due to colinearity between these two covariates, also suggesting an overlap between groups of patients colonized with H. pylori strains that provoke or that do not provoke more intense gastritis. In fact, when the patients were stratified by the cagA status of the H. pylori strain, infection with a cagA-positive bacterium itself was independently and inversely associated with GERD. Furthermore, we also demonstrated that more severe corpus gastritis independently protects against GERD.

**TABLE 2. Results of multivariate analysis with variables associated with GERD in H. pylori-positive and -negative patients**

<table>
<thead>
<tr>
<th>Covariate</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.04</td>
<td>1.02–1.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender</td>
<td>0.60</td>
<td>0.34–1.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Degree of oxyntic gastritis</td>
<td>0.64</td>
<td>0.49–0.84</td>
<td>0.001</td>
</tr>
<tr>
<td>H. pylori infection</td>
<td>0.81</td>
<td>0.41–1.60</td>
<td>0.54</td>
</tr>
</tbody>
</table>

**TABLE 3. Relationship between GERD and variables related to the H. pylori-positive patients studied**

<table>
<thead>
<tr>
<th>Variable</th>
<th>With GERD (n = 49)</th>
<th>Without GERD (n = 142)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD age (yr)</td>
<td>50.4 ± 16.0</td>
<td>42.9 ± 14.8</td>
<td>0.003</td>
</tr>
<tr>
<td>No. of men: no. of women</td>
<td>21:28</td>
<td>54:88</td>
<td>0.61</td>
</tr>
<tr>
<td>cagA status (no. of patients positive: no. of patients negative)</td>
<td>11:34</td>
<td>89:44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (range) oxyntic gastritis score</td>
<td>1.0 (0–5)</td>
<td>2.0 (0–6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Univariate analysis.

**TABLE 4. Results of multivariate analysis with variables associated with GERD in patients infected by H. pylori**

<table>
<thead>
<tr>
<th>Covariate</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.04</td>
<td>1.01–1.07</td>
<td>0.009</td>
</tr>
<tr>
<td>cagA positivity</td>
<td>0.16</td>
<td>0.06–0.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Degree of oxyntic gastritis</td>
<td>0.69</td>
<td>0.48–0.99</td>
<td>0.048</td>
</tr>
</tbody>
</table>

**TABLE 5. Results of multivariate analysis with variables associated with GERD in patients infected by H. pylori**

<table>
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<th>Covariate</th>
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<th>95% CI</th>
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</table>

*Univariate analysis.
Previous studies have demonstrated that carriage of \textit{cagA}-positive strains was inversely related to the severity of the reflux disease, indicating that carriage of \textit{cagA}-positive strains has a protective role against esophageal complications of acid reflux such as Barrett’s esophagus and Barrett’s esophagus complicated by dysplasia or carcinoma (3, 10, 25). Our results, in agreement with those of a recent study (26), extend these findings and show that not only patients with more severe lesions but also those without complications may be protected by infection with \textit{cagA}-positive strains.

Several possible explanations justify the protective effect of infection with \textit{cagA}-positive \textit{H. pylori} strains against GERD. \textit{cagA} is considered a marker of the presence of the \textit{cag} PAI in the \textit{H. pylori} genome. The products of the \textit{cag} PAI genes are associated with increased levels of production of interleukin-8 and more severe gastric lesions (5). Indeed, in the present study we observed that \textit{cagA}-positive strains were associated with higher inflammation scores for the oxyntic mucosa. When the variables were analyzed in a logistic regression model, the variables were analyzed in a logistic regression model, the status remained positive for the oxyntic mucosa. When the variables were analyzed in a logistic regression model, the status remained positive for the oxyntic mucosa. When the variables were analyzed in a logistic regression model, the status remained positive for the oxyntic mucosa.

Although the protective role linked to infection with a \textit{cagA}-positive \textit{H. pylori} strain may be explained by the lower gastric acid output due to the more intense gastric lesions induced by these strains, a direct effect of the more virulent strains may not be ruled out. Thus, one may hypothesize that there is a protective factor(s) linked to a more intense inflammation in the oxyntic mucosa.

In conclusion, we have provided evidence supporting the independent protective roles of \textit{cagA}-positive \textit{H. pylori} strains and the degree of corpus gastritis against GERD. Thus, some protective factor(s) linked to \textit{cagA}-positive \textit{H. pylori} strains other than gastritis should be evaluated, as should a possible host factor(s) linked to a more intense inflammatory response in the oxyntic mucosa.

ACKNOWLEDGMENTS

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REFERENCES


