Age and Severity of Mucosal Lesions Influence the Performance of Serologic Markers in Helicobacter pylori–Associated Gastroduodenal Pathologies

Margarita Camorlinga-Poncé,1 Lourdes Flores-Luna,2 Eduardo Lazcano-Ponce,2 Rolando Herrero,3 Fernando Bernal-Sahagún,4 Juan Miguel Abdo-Francis,4 Jesús Aguirre-García,4 Nubia Muñoz,5 and Javier Torres1

1Unidad de Investigación en Enfermedades Infecciosas, Hospital de Pediatría, Instituto Mexicano del Seguro Social, Centro de Investigación en Salud Poblacional, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico; 2Instituto de Investigación Epidemiológica, San José, Costa Rica; 3Servicio de Patología y Gastroenterología Médica, Hospital General de México, Mexico City, Mexico; and 4Instituto Nacional de Cancerología, Bogotá, Colombia

Abstract

Objective: The course of Helicobacter pylori infection and antibody response to CagA in patients with preneoplastic lesions and gastric cancer has not been thoroughly studied. We aimed to study H. pylori infection and antibody response to CagA in patients with non–atrophic gastritis, preneoplastic lesions, and gastric cancer.

Methods: We studied patients attending one Oncology Hospital and one General Hospital in Mexico City. Diagnosis was based on endoscopy and histopathology in biopsies from six stomach regions. H. pylori infection was assessed by histology and serology, and antibodies against CagA were measured with immunoassay.

Results: We included 618 patients, 368 with non–atrophic gastritis, 126 with precancerous lesions, and 65 with gastric cancer; in addition, 59 patients with duodenal ulcer were studied. Detection of infection and IgG against CagA had a significant increase from non–atrophic gastritis to mild and up to advanced stages of atrophy and metaplasia (P < 0.05), followed by decreased infection and IgG to CagA in patients with gastric cancer (P < 0.05). However, infection and CagA antibodies were associated with young gastric cancer cases. Duodenal ulcer showed a significant association with infection detected by histology and serology, particularly among women, and a trend to associate with IgG to CagA.

Conclusions: This study shows that H. pylori infection and CagA are risk markers for intestinal metaplasia. The prevalence of these risk markers decreases in gastric cancer, probably reflecting that infection decreases after advanced atrophy and metaplasia in the gastric mucosa. State of the disease, age, and sex influence the association of H. pylori infection and IgG response to CagA with gastroduodenal diseases.

Introduction

Helicobacter pylori is a gram-negative bacterium that colonizes the human gastric mucosa early in life and causes chronic inflammation, persisting for decades if untreated (1). The prevalence of H. pylori infection is high (80–90%) in developing countries and lower (20–50%) in developed countries. In Mexico, 50% of the population is infected at 10 years of age and >80% after age 20 years (2, 3).

H. pylori was classified by the WHO as a class 1 carcinogen (4), and numerous studies have confirmed an increased risk for gastric cancer among subjects infected with H. pylori. It is estimated that the infection increases the risk for noncardia gastric cancer more than 6-fold (5). Gastric cancer is the fourth most common cancer and the second leading cause of cancer-associated deaths in the world. Although the incidence of gastric cancer has been steadily decreasing, in some developing nations, such as Chile, it has remained stable during the last 20 years (6), or even slightly increased, like in Mexico (7).

Gastric cancer is a complex, multifactorial disease, with different factors involved. In addition to H. pylori infection, age at acquisition of the infection, genetics of the host, and environmental factors play a role. An association of H pylori virulence factors with an increasing risk for duodenal ulcer and gastric cancer has been observed, particularly with H. pylori strains possessing the cagA pathogenicity island cag PAI (8). H. pylori strains with cagPAI cause a more intense inflammatory response of the gastric mucosa, leading to increased tissue damage and higher risk of developing atrophic gastritis, precancerous lesions, and gastric cancer (9, 10). The CagA protein is the main molecule injected to epithelial cells by the type IV secretion system; it is a highly immunogenic protein which is responsible for many of the cytotoxic and proinflammatory activities which lead to gastroduodenal diseases. An increasing number of studies have shown a close association between CagA antibodies and the development of duodenal ulcer and gastric cancer (8, 11, 12). Several...
studies have shown a correlation between serologic detection of antibodies against CagA and the identification of the infecting \textit{H. pylori} cagA+ strains (13, 14).

It has been observed that when the gastric mucosa loses its characteristic environment, e.g., when atrophy and metaplasia occurs, it no longer offers a suitable niche for \textit{H. pylori} to colonize (15, 16). Thus, \textit{H. pylori} infection is supposed to decline as progression to gastric cancer occurs; and documenting the presence of the infection should become more difficult at these late disease states. If true, it would be expected that the association of \textit{H. pylori} infection with preneoplastic lesions and gastric cancer would tend to become negative. However, it seems that this is not always the case and several studies have reported a significant association between \textit{H. pylori} infection and gastric cancer (8, 17). Some of the above studies used serologic markers, a test which shows evidence of past or current infection.

The natural history of infection and of humoral response to \textit{H. pylori} and to immunogenic antigens such as CagA in patients covering the clinical spectrum from non-atrophic gastritis, preneoplastic lesions, to gastric cancer have not been thoroughly studied. The aim of the present work was to study the prevalence of current or past \textit{H. pylori} infection as well as the antibody response to the virulence-associated CagA protein in patients with a clinical spectrum of gastric lesions which precede the appearance of cancer, from non–atrophic gastritis to gastric cancer; patients with duodenal ulcer were used as a contrast group because active \textit{H. pylori} infections remain in these cases.

Materials and Methods

Patients. We studied patients attending the Gastroenterology Unit of the México General Hospital, Secretaria de Salud and the Oncology Hospital, Instituto Mexicano del Seguro Social, both in Mexico City, from October 1999 to July 2002. We selected patients older than 30 years, who consulted because of gastroduodenal symptoms (General Hospital) or because of a probable gastric cancer (Oncology Hospital), programmed for endoscopy and biopsy for diagnostic purposes. We excluded subjects who had previously received cancer treatment, as well as those who had taken antibiotics, bismuth compounds, proton pump inhibitors, and nonsteroidal anti-inflammatory drugs 2 weeks previous to the study. Those with other severe chronic diseases or the mentally ill were also excluded. The protocol was approved by the Research and Ethics Committee of the Hospital General de México, Secretaria de Salud, and the Oncology Hospital at Instituto Mexicano del Seguro Social. An informed consent letter was signed by all the participants in the study.

Questionnaires. All participants were interviewed using a questionnaire previously validated regarding clinical and demographic characteristics, education level, and smoking and drinking habits.

Clinical and Histopathology Diagnosis. Diagnosis was based on endoscopy findings and on histopathology study. Six biopsies were taken for histopathology and for detection of \textit{H. pylori} infection, four from the antrum, and two from the corpus. Biopsies were placed in 10% formaldehyde saline for histologic examination. Fixed biopsies were stained with Giemsa and H&E, and with periodic acid-Schiff staining when metaplasia was suspected. The presence of \textit{H. pylori} as well as of non–atrophic gastritis, atrophic gastritis, intestinal metaplasia, or dysplasia was documented in each of the six biopsies. Intestinal metaplasia was graded according to the extent of the lesion, mild if it affected <30%, moderate if it affected <60%, and severe if it affected >60% of all the mucosal area examined in the six biopsies per patient. Final diagnosis was that of the most severe histologic lesion in any of the six biopsies analyzed, or based on endoscopy findings in the cases of duodenal ulcer. \textit{H. pylori} infection was considered positive when the bacteria was observed in any of the six biopsies analyzed. A single experienced pathologist analyzed all the specimens.

Serum Samples. A 5 mL blood sample was drawn from each patient; serum was obtained and frozen at −20°C until tested. Serum samples were tested for \textit{H. pylori} infection and for antibodies to CagA as described below.

ELISA for IgG anti-\textit{H. pylori}. IgG antibodies against \textit{H. pylori} whole-cell antigens were tested in sera, using an enzyme-linked immunoabsorbent assay, which was previously validated for use in a Mexican population (18). A pool of whole-cell antigen preparations was obtained from three Mexican strains of \textit{H. pylori} and attached to the plates. Serum samples were tested in a 1:1,000 dilution. Next, a 1:1,000 dilution of antihuman IgG monoclonal antibodies conjugated to alkaline phosphatase (Southern Biotech) was applied. A 1 mg/mL solution of \textit{p}-nitrophenylphosphate was used as a substrate and absorbance was read at 405 nm. All samples were analyzed in duplicate; the final value was given by the average of the two measurements. Patients were considered as seropositive for \textit{H. pylori} infection when ELISA units were ≥1.0 (18).

ELISA for IgG anti-CagA. IgG antibodies against CagA were tested in sera using an ELISA previously validated by us (18). A 0.1 μg/well of recombinant CagA antigen was used (Acambis) and serum at a 1:200 dilution was added. Next, a 1:1,000 dilution of antihuman IgG monoclonal antibodies conjugated to alkaline phosphatase (Southern Biotech) was applied. A 1 mg/mL solution of \textit{p}-nitrophenylphosphate was used as substrate and absorbance was read at 405 nm. Cutoff for CagA seropositivity was defined as a value of ≥1.5 ELISA units (18).

Statistical Analysis. For the analyses, patients with non–atrophic gastritis were considered as controls, whereas those with gastric precancerous or cancerous lesions and those with duodenal ulcer were considered as cases. The general characteristics of the controls and cases were compared using the \textit{t} test and \textit{χ²} tests. The seroprevalence of \textit{H. pylori} infection and the presence of CagA antibodies were determined in each studied disease by groups of age, using the Cuzick test for tendency. Adjusted odd ratios (OR) with 95% confidence intervals (CI) were estimated using polytomous logistic regression. Data were analyzed using the statistical software program Stata 9.0 (Stata Corporation).
Table 1. Demographic characteristics of patients with gastroduodenal lesions

<table>
<thead>
<tr>
<th>Diagnosis*</th>
<th>No. of patients</th>
<th>Mean age (range)</th>
<th>Sex ratio (M/F)</th>
<th>Years of education, mean (SD)</th>
<th>Ever smoking, no. (%)</th>
<th>Ever drinking alcohol, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non–atrophic gastritis</td>
<td>368</td>
<td>48.0 (30-92)</td>
<td>0.7</td>
<td>6.4 (4.5)</td>
<td>146 (39.7)</td>
<td>144 (39.1)</td>
</tr>
<tr>
<td>Atrophic gastritis</td>
<td>14</td>
<td>53.9 (30-75)</td>
<td>0.8</td>
<td>4.9 (4.0)</td>
<td>6 (42.9)</td>
<td>6 (42.9)</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>110</td>
<td>58.1 (30-82)</td>
<td>0.5</td>
<td>4.5 (3.6)</td>
<td>44 (40.0)</td>
<td>42 (38.2)</td>
</tr>
<tr>
<td>Gastric dysplasia</td>
<td>2</td>
<td>76.3 (65-88)</td>
<td>1.0</td>
<td>4.0 (2.8)</td>
<td>2 (100.0)</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>65</td>
<td>61.2 (31-86)</td>
<td>1.6</td>
<td>5.2 (4.2)</td>
<td>37 (56.9)</td>
<td>30 (46.1)</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>59</td>
<td>51.9 (30-80)</td>
<td>1.1</td>
<td>6.0 (4.2)</td>
<td>33 (55.9)</td>
<td>27 (45.8)</td>
</tr>
<tr>
<td>Total</td>
<td>618</td>
<td>51.8 (30-92)</td>
<td>271/347</td>
<td>5.9 (4.3)</td>
<td>268 (43.4)</td>
<td>250 (40.5)</td>
</tr>
</tbody>
</table>

*Patients might have more than one pathology diagnosis in the six biopsies analyzed, but only the most severe was considered.

Results

Table 1 shows the distribution of the study subjects by diagnosis group, age, gender, education, and smoking and drinking habits. A total of 618 patients were included, 368 with non–atrophic gastritis (considered as controls), 126 cases with precancerous lesions (14 with nonmetaplastic atrophic gastritis, 110 with intestinal metaplasia, and 2 with dysplasia), 65 cases with gastric cancer, and 59 with duodenal ulcer. The non–atrophic gastritis patients were younger than all the other case groups included. As expected, patients with gastric cancer were significantly older than controls (mean age of 61 versus 48 years).

More female than male patients were included in the study; this ratio was evident in controls and in cases with intestinal metaplasia, but males were more frequent among gastric cancer cases. Alcohol consumption and smoking were less frequent in controls than in cases of metaplasia and cancer.

Table 2 describes diagnosis of *H. pylori* infection by histology (active infection) and serology (active or past infection), as well as the IgG response to CagA in controls and cases. Detection of *H. pylori* infection by histology in controls and cases with precancerous lesions was lower than detection by serology in ~10%. This difference increased to 25% in cases with gastric cancer; in contrast, both tests had similar results in patients with duodenal ulcer. Antibodies to CagA were more frequent in cases with intestinal metaplasia than in controls and in the other disease groups. It should be noted that contrary to what was expected, the number of patients detected with nonmetaplastic atrophic gastritis was far lower than those with the more severe intestinal metaplasia (which by definition is also atrophic gastritis). In fact, because of the low number of patients with only gastric atrophy and with dysplasia, both groups were excluded from further analyses.

Table 3 shows the distribution of *H. pylori* infection and anti-CagA antibodies in relation to age in each disease group. In patients <45 years of age, the frequency of *H. pylori* infection, as diagnosed by either histology or serology was higher in cases with gastric cancer or with duodenal ulcer than in controls or patients with metaplasia. In contrast, the behavior of the tests was different in patients older than 45 years; infection decreased significantly in cases with cancer, particularly as detected by histology. In contrast, in all age groups older than 45 years, diagnosis of infection (by either histology or serology) was higher in cases with metaplasia and in cases with duodenal ulcer, as compared with controls. The response to CagA was different; it remained higher in all group cases (metaplasia, gastric cancer, or duodenal ulcer) than controls, except for patients older than 65 years in which response declined in patients with gastric cancer or duodenal ulcer but not in cases with metaplasia or in controls.

Figure 1 shows the diagnosis of *H. pylori* infection and CagA antibodies in different lesions, following the natural history for intestinal gastric cancer: non–atrophic gastritis → metaplasia → cancer. Detection of the infection (by serology) and response to CagA significantly increased from non–atrophic gastritis, to mild metaplasia, and to moderate-severe metaplasia (*P* = 0.03 for serology and *P* < 0.01 for IgG CagA, Cuzick test for tendency), and then all three tests decreased in gastric cancer (*P* < 0.001, x² test).

We next analyzed the possible association of *H. pylori* infection and IgG response to CagA with the different disease groups (Table 4). When metaplasia was compared with the control group, infection detected by histology showed a borderline significant OR value (1.5), whereas the association with serology was significant and stronger (OR, 2.0). A positive IgG response to CagA was also significantly associated with metaplasia (OR, 2.4), and this association increased as the magnitude of

Table 2. Diagnosis of *H. pylori* infection and IgG response to CagA in patients with gastroduodenal lesions

<table>
<thead>
<tr>
<th>Diagnosis*</th>
<th>No. of patients</th>
<th><em>H. pylori</em> histology+ (%)*</th>
<th><em>H. pylori</em> serology+ (%)</th>
<th>CagA serology+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non–atrophic gastritis</td>
<td>368</td>
<td>227 (63.1)</td>
<td>270 (73.4)</td>
<td>236 (64.1)</td>
</tr>
<tr>
<td>Atrophic gastritis</td>
<td>14</td>
<td>11 (78.6)</td>
<td>12 (85.7)</td>
<td>9 (64.3)</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>110</td>
<td>76 (69.1)</td>
<td>91 (82.7)</td>
<td>88 (80.0)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>65</td>
<td>26 (41.3)</td>
<td>43 (66.2)</td>
<td>43 (66.2)</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>59</td>
<td>48 (81.4)</td>
<td>52 (88.1)</td>
<td>43 (72.9)</td>
</tr>
<tr>
<td>Total</td>
<td>618</td>
<td>388 (63.8)</td>
<td>469 (75.9)</td>
<td>421 (68.1)</td>
</tr>
</tbody>
</table>

*Patients might have more than one pathology diagnosis in the six biopsies analyzed, but only the most severe was considered.
the IgG response increased (OR, 4.0 for the highest IgG response). When gastric cancer was analyzed, no association was found with infection (neither histology nor serology) or with IgG response to CagA. In contrast, infection, as tested with both histology (OR, 2.8) and serology (OR, 2.9), showed a significant association with duodenal ulcer; in fact, the association increased as the magnitude of the response to H. pylori increased (up to an OR of 3.9). However, only a marginal association was observed with the response to CagA and duodenal ulcer.

Because of the association observed between H. pylori infection and IgG to CagA with metaplasia, we next analyzed this group in more detail, separating the values according to the extension of metaplasia (Table 5). Interestingly, OR values for all three tests, histology and serology for infection, and IgG to CagA increased from non–atrophic gastritis to mild metaplasia and to moderate-severe metaplasia. Thus, OR values were significant for histology (OR, 2.0), and were stronger for H. pylori serology (OR, 2.9) and for IgG to CagA (OR, 4.4) in cases with moderate-severe metaplasia.

We next analyzed the behavior of the tests in the disease groups according to sex and found some differences. In intestinal metaplasia, OR values for IgG to CagA were higher in males (OR, 3.0; 95% CI, 1.2-7.7) than in females (OR, 2.1; 95% CI, 1.1-4.0). In cancer, OR values for H. pylori histology were also lower for females (OR, 0.2; 95% CI, 0.1-0.6) than for males (OR, 0.8; 95% CI, 0.3-1.7). In contrast, in duodenal ulcer, OR values for H. pylori serology were almost five times higher for females (OR, 9.1; 95% CI, 1.2-69.5) than for males (OR, 2.0; 95% CI, 0.7-5.2).

Discussion

The development of gastric cancer takes place during long periods of time; from the initial non–atrophic gastritis lesions to gastric cancer, the progression of the disease takes decades. H. pylori infection is considered to play a major role at the initial steps, causing chronic gastric inflammation and tissue damage, leading to alterations in cell cycle and damage to DNA (19, 20). This chronic aggression to the gastric epithelia eventually causes the appearance of preneoplastic lesions and increases the risk of gastric cancer. The natural history for intestinal type of gastric cancer includes evolution from non–atrophic gastritis to atrophic gastritis to metaplasia to dysplasia and gastric cancer (21); whereas for diffuse gastric cancer, the natural course remains unknown. H. pylori is able to colonize only normal gastric epithelia and when it is altered because of mucosal
damage, such as during the appearance of preneoplastic lesions, the infection tends to disappear. Fading of the infection would alter diagnostic tests, and those documenting active infection such as culture or histology become negative, whereas those testing the memory of infection, such as humoral immune response, decline more gradually (22, 23). Although the last statements are generally accepted, few studies have actually documented these facts. Our work aimed to study the infection and the humoral response in a group of adults attending a general hospital because of gastroduodenal symptoms and a noncancer hospital because of suspicion of gastric cancer. We aimed to capture patients in the different spectra of gastroduodenal lesions associated with H. pylori infection, from non–atrophic gastritis to precancerous lesions to gastric cancer; and additionally tested duodenal ulcer cases, which are also known to be associated with H. pylori infection.

It has been reported that in populations with a high prevalence of H. pylori infection, >50% of adults over 40 years of age will present atrophic gastritis (24, 25), and in areas with a high risk of gastric cancer, intestinal metaplasia can be observed in >40% of adults (26). Although in Mexico, >80% of the adults are infected with H. pylori (2), the population is at low risk for gastric cancer (27); accordingly, in the group of 494 patients with gastroduodenal symptoms studied (without gastric cancer or duodenal ulcer), only 2.8% presented nonmetaplastic atrophic gastritis, whereas cases with intestinal metaplasia were more frequent (22.3%). Although the frequency of nonmetaplastic atrophic gastritis is lower than in previous reports (28), the frequency of intestinal metaplasia is similar to that observed in other populations with a low risk for gastric cancer (29). It could be argued that the low frequency of cases with nonmetaplastic atrophic gastritis is due to a bias of selection for patients with more severe lesions; however, this is unlikely because most of the 494 patients studied presented non–atrophic gastritis (74.5%). It is important to note that in each patient, gastric lesions were studied in six different areas.

### Table 4. H. pylori infection and seroprevalence to CagA as risk factor for precancerous lesions, gastric cancer, and duodenal ulcer

<table>
<thead>
<tr>
<th></th>
<th>Non–atrophic gastritis (controls)</th>
<th>Intestinal metaplasia</th>
<th>Gastric cancer</th>
<th>Duodenal ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>OR (95% CI)*</td>
<td>n</td>
<td>OR (95% CI)*</td>
</tr>
<tr>
<td>H. pylori infection, histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>133</td>
<td>1.0</td>
<td>37</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive</td>
<td>227</td>
<td>1.5 (0.9-2.4)</td>
<td>26</td>
<td>0.5 (0.3-0.9)</td>
</tr>
<tr>
<td>H. pylori infection, serology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>98</td>
<td>1.0</td>
<td>22</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive</td>
<td>270</td>
<td>2.0 (1.3-3.5)</td>
<td>43</td>
<td>0.9 (0.5-1.6)</td>
</tr>
<tr>
<td>H. pylori serology, magnitude</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15-1.24</td>
<td>122</td>
<td>1.0</td>
<td>25</td>
<td>1.0</td>
</tr>
<tr>
<td>1.25-4.91</td>
<td>123</td>
<td>2.4 (1.3-4.3)</td>
<td>29</td>
<td>1.4 (0.7-2.6)</td>
</tr>
<tr>
<td>4.92-18.99</td>
<td>123</td>
<td>2.2 (1.2-4.0)</td>
<td>11</td>
<td>0.6 (0.3-1.4)</td>
</tr>
<tr>
<td>Trend</td>
<td></td>
<td>P = 0.15</td>
<td></td>
<td>P = 0.02</td>
</tr>
<tr>
<td>CagA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>132</td>
<td>2.4 (1.4-4.1)</td>
<td>43</td>
<td>1.4 (0.8-2.5)</td>
</tr>
<tr>
<td>Positive</td>
<td>236</td>
<td>2.7 (1.2-5.6)</td>
<td>48</td>
<td>2.9 (1.3-6.6)</td>
</tr>
</tbody>
</table>

*Patients with non–atrophic gastritis were used as the control group; OR adjusted for sex, age, ever smoking, ever drinking alcohol, and level of education.

### Table 5. Association between H. pylori infection and seroprevalence to CagA according to the extension of intestinal metaplasia

<table>
<thead>
<tr>
<th></th>
<th>Non–atrophic gastritis (controls)</th>
<th>Intestinal metaplasia</th>
<th>Gastric cancer</th>
<th>Duodenal ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>OR (95% CI)*</td>
<td>n</td>
<td>OR (95% CI)*</td>
</tr>
<tr>
<td>H. pylori histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Negative</td>
<td>133</td>
<td>1.0</td>
<td>37</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive</td>
<td>227</td>
<td>1.2 (0.7-2.2)</td>
<td>37</td>
<td>2.0 (1.0-4.0)</td>
</tr>
<tr>
<td>H. pylori serology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>98</td>
<td>1.0</td>
<td>7</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive</td>
<td>270</td>
<td>1.5 (0.8-3.0)</td>
<td>44</td>
<td>2.9 (1.2-6.9)</td>
</tr>
<tr>
<td>CagA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>132</td>
<td>1.0</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive</td>
<td>236</td>
<td>1.5 (0.8-2.9)</td>
<td>45</td>
<td>4.4 (1.8-11.0)</td>
</tr>
</tbody>
</table>

*Patients with non–atrophic gastritis were used as the control group; OR adjusted for sex, age, ever smoking, ever drinking alcohol, and level of education.
different regions of the stomach by an experienced pathologist, increasing the possibility to detect any lesion. Thus, it seems that in contrast with other populations, in our population, in *H. pylori*-infected and symptomatic adults over 40 years, most patients with nonmetaplastic gastric atrophy evolve shortly to intestinal metaplasia.

Because of the low number of cases with nonmetaplastic atrophic gastritis and dysplasia in the group with preneoplastic lesions, only patients with intestinal metaplasia could be further analyzed. Thus, we studied the behavior of the *H. pylori* tests exclusively in patients with intestinal metaplasia and cancer, with patients with non-atrophic gastritis as the control group, as well as in patients with duodenal ulcer. We first asked if age influenced diagnosis and found that results were different in young adults. In patients <45 years with cancer, infection, as detected by both histology and serology, was higher than in the control group, whereas the opposite was found in older patients, and this was more evident with the histology test. The results that in young cancer patients, there is more intact epithelium suitable for *H. pylori* colonization and the association with infection is still evident. This is an observation which has been poorly documented previously; although in studies in young adults (<40 years) with cancer (30), and in cases with less invasive and small-sized lesions (31), a strong association with infection was reported, also suggesting the disappearance of *H. pylori* infection due to extended mucosal atrophy. In contrast to the observation in cancer patients, in cases with intestinal metaplasia, the infection remained higher than in the control group at all age groups. Thus, in patients with intestinal metaplasia, detection of *H. pylori* infection showed significant OR values (Table 4). Similarly, in patients with duodenal ulcer, *H. pylori* infection was also a significant risk factor in all age groups, confirming that in these groups, infection remains significantly higher than in patients with non-atrophic gastritis at all age groups.

The IgG response to CagA was more frequent in gastric cancer patients up to 64 years old; after which age, the response became lower than in the control group; whereas in patients with intestinal metaplasia, the antibody response remained higher than controls at all age groups. This was consistent with the OR values we obtained (Table 4); thus, the response to CagA was significantly associated with risk of intestinal metaplasia and only marginally to gastric cancer and duodenal ulcer. In fact, in intestinal metaplasia, as the magnitude of the response to CagA increased, the OR values also increased, and this association was not observed in the other disease groups. These results would suggest that CagA is a stronger risk factor for precancerous lesions (hence, for cancer) than for duodenal ulcer (32).

Based on the observations, we studied infection and CagA response in patients with intestinal metaplasia in more detail according to the extension of the lesions. We found that the strength of the association with *H. pylori* infection (either with histology or serology) tended to increase from non-atrophic gastritis to mild and to moderate-severe metaplasia; and then decreased from moderate-severe metaplasia to cancer. These results confirm the risk of infection with CagA+ *H. pylori* for the development of precancerous lesions and the fading of the infection as gastric cancer appears (28).

Finally, we analyzed if sex had any influence on the association of disease with *H. pylori* infection or IgG response to CagA. For intestinal metaplasia and gastric cancer, response to CagA had higher OR value for males than for females; an observation which is in accordance with the higher prevalence of gastric cancer among men (33). In contrast, in duodenal ulcer, serology for *H. pylori* infection was much higher for females (OR, 9.1; 95% CI, 1.2-69.3) than for males (OR, 2.0; 95% CI, 0.7-5.2); this difference has not been reported previously. Thus, our results suggest that infection and CagA might represent different risk factor for gastroduodenal diseases in males and females; these results need to be confirmed with a higher number of patients.

We acknowledge that the study has some limitations, we included only patients seeking medical attention because of gastroduodenal symptoms and thus our control group (non–atrophic gastritis) may be more likely to have *H. pylori* markers; this fact could attenuate the association with disease groups. Also, the sample size of some of the analyzed groups was reduced, resulting in a poor statistical strength. Future studies are needed to confirm the gradual loss of *H. pylori* as integrity of the gastric mucosa is reduced by extended atrophy, ideally by prospective studies. Although we assessed infection with *H. pylori* cagA+ strains indirectly by measuring antibodies against CagA, previous studies have shown a close correlation between the detection of antibodies to CagA and the detection of the cagA gene in the isolated strain (13, 14). In fact, in previous studies in Mexican adults with peptic ulcer, serologic response to CagA was 83.7% (3), whereas cagA+ *H. pylori* strains were detected in 81% of the patients (34).

In summary, the results of our study detail the increased association of *H. pylori* infection and IgG response to CagA in patients from non–atrophic gastritis to mild metaplasia to moderate-severe metaplasia, and then the decreased association of *H. pylori* infection from metaplasia to gastric cancer; showing that the role of *H. pylori* infection and CagA as risk factors for gastric cancer is more clear when measured in metaplasia than in gastric cancer cases. However, in young adults with cancer, the role of *H. pylori* infection and CagA as risk factors is still evident. Thus, state of the disease, age, and sex influence the association of *H. pylori* infection and of IgG response to CagA with gastroduodenal diseases.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**


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Margarita Camorlinga-Ponce, Lourdes Flores-Luna, Eduardo Lazcano-Ponce, et al.


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