CagA status and *Helicobacter pylori* eradication among dyspeptic patients

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ABSTRACT

**Hypothesis:** Triple therapy seems more effective in curing *Helicobacter pylori* infection in patients with peptic ulcer than in those with non-ulcer dyspepsia. It has been suggested that this difference depends on the expression of CagA protein that is more frequent in the former. The objective of this study was to investigate a potential association between serum CagA positivity, severity of gastric mucosal inflammation and eradication success among peptic ulcer and non-ulcer dyspepsia patients.

**Material and Method:** Patients undergoing upper gastrointestinal endoscopy for investigation of dyspepsia at the Department of Gastroenterology, Hospital Vera Cruz, between March 2000 and March 2001 were screened. *H. pylori* positive patients, as diagnosed by rapid urease test and histology were included. Severity of gastric mucosal inflammation was determined and serum CagA positivity was assessed using a commercially available ELISA assay prior to *H. pylori* 7-day eradication therapy with lansoprazole, clarithromycin and amoxicillin (30 mg, 500 mg and 1 g b.i.d., respectively). Eradication success was determined 8-24 weeks following completion of therapy.

**Results:** Seventy-four patients were included in the study (mean age 40.8, range 18-67, female = 28). CagA positivity was observed in 48% of patients. Gastroduodenal peptic ulceration was found in 24% of patients. Serum CagA positivity was significantly higher among peptic ulcer patients (62.5%), while CagA negativity was significantly higher among non-ulcer dyspepsia patients (67.5%). Lymphocyte and eosinophil infiltration was significantly higher among *CagA +* patients, despite being comparable when distributed among peptic ulcer and non-ulcer dyspepsia patients. Eradication was successful in 93.2% of patients, regardless of CagA status on a per protocol analysis. Based on a per protocol analysis, eradication success was comparable among peptic ulcer and non-ulcer dyspepsia patients, regardless of CagA status.

**Conclusion:** Our results support the concept that CagA positivity is associated to peptic ulcer disease and to a higher severity of lymphocyte and eosinophil infiltration. Efficacy of treatment eradication of *H. pylori* may not be affected by serum CagA status.

CagA Y ERRADICACIÓN DE *HELICOBACTER PYLORI* EN PACIENTES CON DISPEPSIA

**Objetivo:** El tratamiento triple parece tener una eficacia mayor en la curación de la infección por *Helicobacter pylori* en los pacientes con úlcera péptica, en comparación con los pacientes que presentan dispepsia de origen no ulceroso. Se ha señalado que esta diferencia depende de la expresión de la proteína CagA, que es más frecuente en los primeros. El objetivo de este estudio ha sido la investigación de una posible asociación entre la positividad sérica para CagA, la intensidad de la inflamación de la mucosa gástrica y el buen resultado de la erradicación de la infección por *H. pylori* en pacientes con úlcera péptica y en pacientes con dispepsia de origen no ulceroso.

**Material y método:** El estudio se realizó en pacientes atendidos entre marzo de 2000 y marzo de 2001 en el Departamento de Gastroenterología, Hospital Vera Cruz; en todos los pacientes se había indicado la realización de endoscopia gastrointestinal alta como parte del estudio de un cuadro de dispepsia. Los participantes presentaron positividad para *H. pylori*, tanto en la prueba rápida de la ureasa como en el estudio histológico. Se determinó la intensidad de la inflamación de la mucosa gástrica y se evaluó la positividad sérica para CagA mediante el uso de una técnica de ELISA comercializada, todo ello antes de la administración de un ciclo de tratamiento de 7 días para la erradicación de *H. pylori* con lansoprazol, claritromicina y amoxicilina (30 mg, 500 mg y 1 g cada 12 h, respectivamente). El resultado de la erradicación se estableció a las 8-24 de semanas de la finalización del tratamiento.
RESULTADOS: En el estudio participaron 74 pacientes (edad media, 40.8 años; rango, 18-67 años; número de mujeres, 28). Se detectó positividad para CagA en el 48% de los pacientes. El 54% de ellos presentaba úlcera péptica gastrroduodenal. La positividad sérica para CagA fue significativamente mayor en los pacientes con úlcera péptica y en los pacientes con dispepsia de origen no ulceroso (67.7%). La intensidad de la infiltración por linfocitos y eosinófilos fue significativamente mayor en los pacientes con positividad para CagA, a pesar de que dicha intensidad fue similar entre los pacientes con úlcera péptica y en los pacientes con dispepsia de origen no ulceroso. En un análisis de protocolo individualizado, la erradicación de la infección tuvo éxito en el 92.2% de los pacientes, con independencia de la positividad o negatividad para CagA. En este tipo de análisis, el buen resultado respecto de la erradicación de la infección fue comparable en los pacientes con úlcera péptica y en los pacientes con dispepsia de origen no ulceroso, con independencia de la positividad o negatividad para CagA. Conclusiones: Nuestros resultados apoyan la posibilidad de que la positividad para CagA esté asociada a la enfermedad ulcerosa péptica y a una intensidad mayor de la infiltración por linfocitos y eosinófilos. La eficacia del tratamiento de erradicación de la infección por H. pylori puede no estar influída por la positividad o negatividad sérica para CagA.

OVERVIEW
Helicobacter pylori eradication is crucial in the treatment of peptic ulcer disease, and plays a key role in modifying the natural course of peptic ulcer disease.27 However, the benefits of H. pylori on symptom control is controversial, especially in patients with non-ulcer dyspepsia.28 The reduction in ulcer recurrence observed following eradication of the bacteria improves symptoms and quality of life of peptic ulcer patients, potentially reducing the risk of further ulcer complications. On the other hand, this may not be the case in functional dyspepsia patients.28 It has been proposed that successful H. pylori eradication is more frequently observed in patients diagnosed with peptic ulcer compared to non-ulcer dyspepsia.29 It has been observed that a bacterial virulence factor such as CagA gene positivity is more often found in peptic ulcer patients, while most non-ulcer dyspeptic patients are CagA-negative.30 Therefore, an association between CagA positivity, peptic ulcer and effective H. pylori eradication may take place. The present study was designed to investigate whether there would be an association between CagA seropositivity, severity of gastric inflammation, peptic ulcer disease and the H. pylori response to triple therapy.

MATERIAL AND METHOD
Subjects
Patients (both sexes) undergoing upper gastrointestinal endoscopy for evaluation of dyspepsia at the Department of Gastroenterology, Hospital Vera Cruz, between March 2000 and March 2001 were considered for study participation. The inclusion criteria was the presence of H. pylori detected by histology (HE and Giemsa staining) as well as by the rapid urease test. Patients with previous gastric surgery, gastric cancer, chronic liver disease, known biliary disease, pancreatic disease, past history of upper gastrointestinal bleeding, previous H. pylori eradication treatment or antibiotics use 4 weeks prior to study entry were excluded. Written informed consent was obtained from all patients prior to entering the study, and the protocol was approved by the Hospital Vera Cruz Ethics Committee, in accordance with the Declaration of Helsinki.

Seventy four patients (M = 46; F = 28, mean age 40.8 years old, range 18-67) agreed to participate in the study and were included in the study protocol. Twenty two patients were current smokers (29.7%). Upper gastrointestinal endoscopy following an overnight fast using a videoscope (Pajunen 200 HR, Tokyo, Japan) was employed for detection of gastroesophageal peptic lesions and collection of gastric biopsies (2 antrum and 2 corpus) for determination of H. pylori status.31 Endoscopic and histologic gastritis were grade according to the Sydney System. A numeric score was employed to assess the degree of mucosal inflammation, lymphocytic, eosinophilic, and neutrophilic infiltration, as well as the presence of lymphoid follicles before receiving eradication treatment. Histological assessment was performed by a pathologist blinded to endoscopy and CagA positivity. Serum CagA status was assessed in all patients prior to eradication therapy using an Elisa assay (CagAssay, Biomerics, City, Country). Specificity and sensitivity of the assay used to detect CagA seropositivity were performed by testing eighty control sera from an adult population and 40 consecutive patients evaluated by the endoscopy method. CagA positivity was found in five normal individuals. Among twenty nine patients with positive serology on endoscopy, patients were also positive for CagA while in seventeen patients negative for the endoscopy method, only one showed positivity for the presence of CagA.

The patients were subjected to eradication therapy consisting of a 7-day twice daily oral administration of lanospazol 80 mg, amoxicillin 1 g and clarimoxycin 500 mg (Pylorix®, Medley Indústria Farmacêutica, Curitiba, State, Brazil). H. pylori eradication was confirmed when both tests (histology and rapid urease) were negative, assessed 8–14 weeks following termination of eradication therapy. After completing the 7-day triple therapy, the patients did not take any antibiotics or proton-pump inhibitors.

Statistics
The relationship (per protocol and intention-to-treat analysis) between CagA status, presence of peptic ulcer, and the effect of the eradication treatment among the patients studied was assessed using Fisher’s exact test. The severity of gastritis among CagA positive and negative patients was compared using unpaired t-test. Differences were considered to be significant when p < 0.05.

RESULTS
Pepitic ulceration (gastric ulcer [n = 5], duodenal ulcer [n = 35]) was detected in 40 patients (54%; 95% CI = 42.1-65.7%, M = 27, F = 13, mean age 40.1 years old, range 18-67), 27.5% being smokers. Thirty-four patients had a normal mucosa or non-erosive endoscopic gastritis (46%, M = 19, F = 15, mean age 41.7 years old, range 20-65), 32% smokers. Thirty six patients (48.6%, 95% CI = 36.8-60.5%, M = 25) were found to be CagA positive on the Elisa assay. A significantly (p < 0.05) higher proportion of peptic ulcer patients had serum CagA positivity: 25/40 (62.5%, 95% CI = 45.8-77.3%), while serum CagA positivity was significantly (p = 0.05) lower among non-ulcer dyspeptic patients: 11/34 (32.3%, 95% CI = 17.4-50.5%). Fifteen patients (peptic ulcer = 10, non-ulcer dyspepsia = 5) refused to be subjected to a repeat endoscopy in order to assess H. pylori eradication. Therefore, eradication was determined in 59 patients (79.7%, 95% CI = 68.8-88.2%). Eradication was successful in 90-95%, on a per protocol analysis, regardless of CagA status (table I), and comparable among peptic ulcer and non-ulcer dyspepsia patients (table II). Histological assessment was performed on samples collected from patients that had H. pylori erad-
cation tested. Therefore, analysis of gastric mucosal inflammation, leukocyte infiltration and development of lymphoid follicles was investigated in 79.7% of patients. The severity of mucosal inflammation was comparable (p = 0.06) among serum CagA positive and negative patients. However, a significantly (p < 0.05) higher eosinophil and lymphocyte mucosal infiltration was observed in CagA positive patients (fig. 1). Similar neutrophil infiltration and lymphoid follicle development was observed among both groups (fig. 1). No differences were observed on the histological severity score when peptic ulcer and non-ulcer dyspeptic CagA positive and negative were analyzed.

**DISCUSSION**

A lower eradication rate has been demonstrated among _H. pylori_ positive, non-ulcer dyspeptic patients\(^1\). Several factors may contribute to this observation, including a reduced severity of gastric mucosal inflammation, likely related to a «less virulent» bacteria. It has been proposed that VacA\(^16-19\) triple therapies 34 eradication (per protocol and intention-to-treat) among CagA+ and CagA– patients.

<table>
<thead>
<tr>
<th>Peptic ulcer</th>
<th>CagA+ (n, %)</th>
<th>CagA– (n, %)</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 (62.5)</td>
<td>15 (37.5)</td>
<td>40</td>
</tr>
<tr>
<td>Non-ulcer dyspepsia</td>
<td>34 (67.7)</td>
<td>23 (42.3)</td>
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<tr>
<td>Total</td>
<td>38 (48.6)</td>
<td>38 (51.4)</td>
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<table>
<thead>
<tr>
<th>Peptic ulcer</th>
<th>CagA+ (n, %)</th>
<th>CagA– (n, %)</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30/32 (92.7)</td>
<td>30/36 (83.3)</td>
<td>60</td>
</tr>
<tr>
<td>Non-ulcer dyspepsia</td>
<td>25/27 (92.5)</td>
<td>25/30 (83.3)</td>
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<tr>
<td>Total</td>
<td>55/59 (93.2)</td>
<td>55/71 (74.3)</td>
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\(^* p < 0.05\) versus CagA–; \(^* p < 0.05\) versus CagA+.

**REFERENCES**


