Association between *Helicobacter pylori* infection and serum pepsinogen concentrations in gastroduodenal disease

Kyoichi Matsumoto, Noboru Konishi, Masato Ohshima, Yoshio Hisa, Emi Kimura, Tomohiro Samori

Abstract

**Aim**—To investigate the association between *Helicobacter pylori* infection and serum pepsinogen (PG)1 and 2 concentrations in various gastroduodenal diseases.

**Methods**—Serum PG1 and 2 concentrations and antibodies to *H pylori* were measured by enzyme linked immunosorbent assay (ELISA); gastric mucosal pH was assessed and urease activity in biopsy tissue was determined. A comparison of the ELISA and urease test results permitted division of the cases into positive, false positive, false negative and negative categories for control, gastritis, and ulcer groups.

**Results**—The gastric mucosal pH and serum PG2 in cases positive for *H pylori* were significantly increased in ulcer and gastritis cases compared with *H pylori* negative cases. Similar tendencies were observed for the false positive and false negative categories.

**Conclusions**—A positive ELISA reaction for antibodies and an increased serum PG2 concentration are reliable indicators of *H pylori* infection.

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Keywords: *Helicobacter pylori*, pepsinogen, ELISA, gastroduodenal disease.

Since it was first isolated and cultured from the human gastric antrum by Warren and Marshall,1-3 several studies have shown an association between gastroduodenal ulcers and *Helicobacter pylori*. The organism is currently thought to be a major cause of gastric mucosal lesions.

Pepsinogen (PG), which is classified into groups 1 and 2 according to immunological cross reactions, is an enzyme synthesised in the gastric mucosa. Samloff et al4 identified seven isoforms using agar electrophoresis. Serum PG is thought to reflect the gastric mucosal state, with increased PG concentrations reported to be closely associated with various gastric lesions.7-11 Serum PG1 concentrations are raised in children with *H pylori* associated gastritis,12 and correlate with the degree of gastritis in infected adults.13 Recently, it was shown that an asymptomatic Japanese population14 and dyspeptic patients15 positive for *H pylori* had significantly higher PG1 and 2 concentrations and a significantly lower PG1:2 ratio than patients who tested negative for *H pylori*. A model has been proposed in which *H pylori* secrete substances which mediate inflammation that is beneficial to the organism but ultimately deleterious for the host; in addition to tissue damage, this inflammation also affects gastric secretory function.16 In fact, *H pylori* also increases serum gastrin concentrations, although the values return to normal after the organism has been eradicated.17-20 The most accurate method for detecting *H pylori* is isolation and identification at the time of endoscopic examination, or breath test using 14C-urea.21 However, the first imposes a great burden on patients and the second cannot readily be performed because of the use of a radioisotope. While the enzyme linked immunosorbent assay (ELISA) for the detection of *H pylori* antibodies in the blood has been frequently reported as a simpler screening method,22-26 the results have not always agreed with those obtained from culture and urease breath testing.

We previously established a model using polyclonal and monoclonal antibodies against PG 1 and 2 and investigated associations between serum PG concentrations and gastroduodenal diseases.27-28 To clarify the gastric mucosal state of patients showing false positive or false negative results for ELISA, we evaluated the dissociation between serum antibodies and urease activity in gastric mucosal biopsy specimens by comparison with the gastric mucosal pH and the serum PG1 and 2 concentrations to evaluate their influence.

**Methods**

Material was obtained from the following sources: globulin-free bovine serum albumin (BSA); o-phenylenediamine (OPD) from Sigma Chemical Co. (St Louis, Missouri, USA); porcine pepsin and horseradish peroxidase (grade 1, HRP) from Boehringer Mannheim (Mannheim, Germany); 96-well micro plates from Nunc (Roskilde, Denmark); Sephacryl S-300 from Pharmacia Fine Chemicals (Uppsala, Sweden); Brucella broth from Difco Laboratories (Detroit, Michigan, USA); Gas Pack system from BBL (Cockeysville, Maryland, USA); peroxidase conjugated anti-human IgG from MBL (Nagoya, Japan); α-octyl-glycoside and other chemicals from Wako Pure Chem. Inc. (Osaka, Japan)
After informed consent had been obtained blood samples were taken from 169 patients (70 men, 99 women; mean age 54 years, range 14–83 years). Endoscopic examination was performed using an Olympus GIF-XQ endoscope.

Following the method of Kohli et al., two biopsy specimens (from the antrum and body mucosa) were suspended into 0.1% phenol red solution containing 10% urea. When the colour of the dye solution changed to red within 24 hours, the specimens were judged to be \textit{H. pylori} positive.

Serum samples were examined for \textit{H. pylori} antibodies by ELISA using the high molecular weight, cell associated protein (HM-CAP) antigen, as described before.\textsuperscript{24} Cultures of \textit{H. pylori} (NCTC 11638) were streaked on Brucella/10% horse blood agar plates and incubated at 37°C for four days in anaerobic jar plates under microaerobic conditions (Gas Pack system without a catalyst). The cells were washed twice by centrifugation (8000 rpm for 20 minutes) and resuspended in phosphate buffered saline (PBS). The bacterial pellet was then suspended in a 1% solution of n-octylglycoside in PBS, pH 7.2. After a 20 minute incubation at room temperature the cells were centrifuged at 15 000 rpm for 20 minutes. The dialysate was centrifuged at 18 000 rpm for 20 minutes. The supernatant fluid was placed on a Sephacryl S-300 column (2.5 x 100 cm) and eluted with PBS. The optical density at 280 nm and urease activity were determined for this 5 ml fraction. The fractions showing urease activity were pooled, then absorbed onto 96-well microenzym immunoassay plates (antigen: HM-CAP) overnight at 10 μg/ml in PBS. After the plates were washed three times with PBS containing 0.05% Tween 20, each well was blocked with 1% BSA in PBS. Then, 100 μl of specimen, diluted 1 in 500 with PBS containing 2 mmol/l EDTA-2Na and 100 μg/l BSA, were added to each well and allowed to react at 37°C for one hour. After the plates were washed again, 100 μl peroxidase labelled anti-human IgG antibody was added and incubated at 37°C for another hour. After washing three times 100 μl substrate solution (pH 4.8; containing 20 mmol/l citric acid-sodium phosphate buffer, 0.03% OPD, and 0.003% H₂O₂) was added. After incubation at 37°C for 30 minutes, 100 μl 2 N sulfuric acid was added to stop the reaction. Absorbency was determined at λ₁ = 492 nm and λ₂ = 660 nm. The cut off for \textit{H. pylori} positivity was determined by the values for culture negative patients. In this study four categories were applied for evaluation of \textit{H. pylori} infection as follows: positive, antibody to \textit{H. pylori} (+) and urease activity (+); negative, absence of antibody to \textit{H. pylori} (−) and urease activity (−); false positive, antibody to \textit{H. pylori} (+) but not urease activity (−); and false negative, no antibody to \textit{H. pylori} (−) but antibody to urease activity (+).

Serum PG1 and 2 concentrations were determined according to the technique developed in our laboratory, which has been reported before.\textsuperscript{23} Briefly, each well of a 96-well microtitreplate was filled with 0.2 ml of PG monoclonal antibodies diluted with PBS to 20 mg/l (2F5 for the determination of PG 1, and 2D5 for the determination of PG 2) and left standing at 4°C overnight for absorption. After the plate was washed three times with PBS containing 0.05% Tween 20, the wells were blocked with 5% BSA in PBS. After 1 in 5 dilution with peroxidase labelled PG monoclonal antibodies (PG1: PG2: 8G2), prepared according to the method of Yoshikawa et al.,\textsuperscript{25} 0.2 ml aliquots of specimen were added to each well, and incubation carried out at 37°C for one hour. After washing with PBS three times 0.2 ml substrate solution (pH 4.8; containing 20 mmol/l citric acid-sodium phosphate buffer, 0.03% OPD and 0.003% H₂O₂) was added and allowed to react at 37°C for 30 minutes. Subsequently, 0.05 ml 4 N sulphuric acid was added to stop the reaction. Absorbency was determined at λ₁ = 492 nm and λ₂ = 660 nm. Serum PG1 and 2 concentrations were determined by comparison with standard curves generated simultaneously.

Statistical analysis of the results was performed using Student’s \textit{t} test.

\textbf{Results}

Data on serum PG1 and 2 concentrations, the PG1:2 ratio, and positivity for \textit{H. pylori} antibody and urease activity in gastric biopsy material are shown in table 1. The positive cases comprised 31.3% (10/32), 55.4% (41/74), and 74.6% (47/63) in the control, gastritis, and ulcer groups, respectively. The respective figures for negative cases were 50% (16/32), 17.6% (13/74), and 6.4% (4/63). Of the 52 cases negative for \textit{H. pylori} antibody in the gastric ulcer, gastritis, and control groups, 19 were false negatives. The ELISA false negative ratio (false negative cases: negative cases) was significantly lower in the control (11.1%, 2/18) group than in the ulcer (36.6%, 10/28), gastritis (45.0%, 12/25), and control groups. Twenty three of 117 subjects positive for \textit{H. pylori} antibodies gave a negative urease breath test. The false positive ratios (false positive case:positive case) were 6.3% (2/32) in the control, 13.5% (7/52) in the ulcer, and 16.3% (8/49) in the gastritis groups. The differences were not significant. The gastric mucosal pH and serum PG1 and PG2 concentrations were higher in the \textit{H. pylori} positive than in the negative cases in the same disease categories (tables 1 and 2). In particular, the gastric mucosal pH and serum PG2 in positive cases were significantly increased in all three groups, without any disease association. The gastric mucosal pH and serum PG2 in the ulcer and gastritis false positive cases were also significantly increased. Serum PG2 concentrations in the gastritis and control false negative cases were significantly higher than in the \textit{H. pylori} negative subjects. The ratios for serum PG2 and gastric mucosal pH were significantly higher than the ratios for serum PG1 (table 2). When the three groups (169 subjects) were subdivided into gastric or duodenal ulcer, erosive, metaplastic or atrophic gastritis, there were no significant differences in the serum PG1 and 2 concentrations or in the
**Table 1** Infectious state of *H pylori* and serum pepsinogen 1 and 2

<table>
<thead>
<tr>
<th>Disease</th>
<th>Group</th>
<th>Antibody to <em>H pylori</em></th>
<th>Urease activity</th>
<th>No of cases</th>
<th>Age</th>
<th>Gastric mucosal pH</th>
<th>Serum PG1 (ng/ml)</th>
<th>Serum PG2 (ng/ml)</th>
<th>PG1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>65</td>
<td>51</td>
<td>51.3 (14.5)</td>
<td>2.6 (2.2)</td>
<td>45.6 (16.1)</td>
<td>20.0 (9.4)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>-</td>
<td>47</td>
<td>51</td>
<td>13.7 (7.2)</td>
<td>2.6 (2.1)</td>
<td>48.5 (16.4)</td>
<td>21.8 (9.4)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+</td>
<td>-</td>
<td>7</td>
<td>62</td>
<td>0.0 (19.8)</td>
<td>4.0 (3.2)</td>
<td>39.5 (12.8)</td>
<td>17.4 (8.1)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>5</td>
<td>46.8</td>
<td>15.0 (5.1)</td>
<td>1.9 (1.1)</td>
<td>34.7 (7.4)</td>
<td>15.9 (6.6)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
<td>+</td>
<td>4</td>
<td>38.8</td>
<td>18.8 (5.1)</td>
<td>1.2 (0.5)</td>
<td>35.8 (16.9)</td>
<td>9.1 (3.8)</td>
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<tr>
<td></td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>57.7</td>
<td>13.2 (5.1)</td>
<td>4.0 (2.6)</td>
<td>37.9 (22.7)</td>
<td>21.2 (12.7)</td>
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<tr>
<td>Gastritis</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>41</td>
<td>58.8</td>
<td>12.6 (5.1)</td>
<td>4.5 (2.4)</td>
<td>40.4 (19.6)</td>
<td>22.1 (7.3)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>-</td>
<td>12</td>
<td>58.8</td>
<td>7.8 (5.1)</td>
<td>4.8 (3.0)</td>
<td>34.1 (23.7)</td>
<td>21.5 (10.2)</td>
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<td>3</td>
<td>-</td>
<td>+</td>
<td>13</td>
<td>50.8</td>
<td>18.4 (5.1)</td>
<td>1.6 (1.6)</td>
<td>29.1 (12.7)</td>
<td>8.6 (2.5)</td>
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<tr>
<td></td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>45.7</td>
<td>17.8 (5.1)</td>
<td>2.3 (2.0)</td>
<td>38.9 (16.6)</td>
<td>17.7 (12.2)</td>
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<tr>
<td>Control</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>10</td>
<td>52.1</td>
<td>16.3 (5.1)</td>
<td>3.3 (2.0)</td>
<td>46.0 (18.7)</td>
<td>28.0 (15.3)</td>
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<td>+</td>
<td>-</td>
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<td>19.4 (12.8)</td>
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<td>3</td>
<td>-</td>
<td>+</td>
<td>2</td>
<td>44.5</td>
<td>21.9 (5.1)</td>
<td>4.3 (4.2)</td>
<td>37.0 (6.1)</td>
<td>23.1 (4.1)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>41.7</td>
<td>20.0 (5.1)</td>
<td>1.5 (0.9)</td>
<td>34.1 (11.7)</td>
<td>10.1 (6.1)</td>
</tr>
</tbody>
</table>

*Significantly different from group 4 (antibody (+), urease (-)) by Student's t test (p < 0.05).

*Significantly different from group 4 (antibody (+), urease (+)) by Student's t test (p < 0.005).

*Significantly different from group 4 (antibody (+), urease (+)) by Student's t test (p < 0.001).

PG1:2 ratio except for decreased PG1 and PG2 in the atrophic gastritis cases (data not shown).

**Discussion**

It has been reported that the titre of antibodies directed against *H pylori* in serum can be used as a non-invasive indicator of the presence of *H pylori* infection and gastritis. Goodwin et al. found a higher titre in active chronic than in inactive chronic gastritis and Newell et al. noted the same tendency in severe gastritis cases. In the present study the prevalence of *H pylori* positive cases was higher in the gastroduodenal disease categories than in the controls. The titre of antibodies directed against *H pylori* in the serum significantly correlated with gastric mucosal pH and serum PG1 and 2. However, ELISA false negative results made up 36.5% (19/52) of the negative cases, with higher incidences in the ulcer and gastritis groups than in the controls. We therefore conclude that detection of the antibodies to *H pylori* using ELISA is reliable for asymptomatic subjects, but not for patients with gastroduodenal diseases. However, serum PG2 concentrations in the false negative cases were higher than in the *H pylori* negative cases, and very similar to those in the *H pylori* positive cases. Therefore, the serum PG2 concentration may be a useful biomarker for infection when ELISA results are negative.

The ELISA is a simple and easy method for the detection of serum antibodies and diagnosis of infectious disease. As *H pylori* and other *Campylobacter* species share common antigens, many serum antibodies cross-react with different species. Recently, the use of purified *H pylori* antigens has been reported to improve the specificity, but ELISA findings may still be partly inconsistent with results of culture or the urease activity test. In the present study negative results observed in gastroduodenal diseases might have had increased gastric mucosal pH and serum PG2 as a result of previous *H pylori* infections, with antibodies no longer present, or because of early stage infection before generation of an immune response. Clearly, assays for serum antibodies to *H pylori* are by themselves insufficient for diagnosis of infection in dyspeptic patients.

Hunter et al. have reported that eradication of infection results in significantly decreased concentrations of PG1 and PG 2, with at least a 25% drop in PG2 after completion of treatment in 82% of subjects. Successful treatment of *H pylori* infection resulted in only an approximately 20% reduction of the *H pylori* antibody titre in 65% and 89% of cases after three and six months of treatment, respectively, indicating that serum PG2 is the more sensitive marker. Our results provide further support for this conclusion.

Raised PG1 and 2 concentrations have been observed in asymptomatic or dyspeptic individuals positive for *H pylori* antibodies than in their antibody negative counterparts. However, serum PG1 did not differ between false negative and negative cases in the present study, in clear contrast to the findings for PG2. This might be due to the fact that the gastric antrum, which produces PG2, is where *H pylori* mainly resides.

Serum PG concentrations are considered to be reliable indicators of chronic atrophic gastritis and ulceration. However, the prevalence of *H pylori* infection was higher in ulcer and gastritis cases than in the controls. The increase in serum PG1 and 2 in patients with gastroduodenal diseases in previously published reports might have been due to the presence of bacteria.
H. pylori infection is a major cause of gastroduodenal disease and its eradication results in rapid improvement. Therefore, optimal treatment in cases with H. pylori infection requires not only an H2-receptor antagonist but also measures against the organism itself. From this point of view, H. pylori infection is the key to prevention or cure of mainly gastroduodenal problems. Several methods have been developed for the detection of H. pylori. CLO tests for detection of H. pylori urease activity and histological examination require endoscopy, which is known to be valuable for clinical purposes, but cumbersome and expensive. Breath test with isotopes of urea is inconvenient, costly, time consuming, and results in undesirable exposure to radiation. Measurement of antibodies to H. pylori and serum PG2 in blood samples is less expensive and non-invasive, but not an accurate sampling technique. The combination of these two parameters is a useful serological approach for the detection of H. pylori infection.

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