Papers

Diagnosis of Helicobacter pylori infection by specific gastric mucosal IgA and IgG pylori antibodies


Abstract

Aims—To investigate the diagnostic value of mucosal IgA and IgG Helicobacter pylori antibodies.

Methods—The study population comprised 209 consecutive patients with severe dyspeptic complaints referred for upper gastrointestinal endoscopy. A positive culture or histological identification of H pylori in gastric biopsy specimens, or both, were used to confirm infection. Specific IgA and IgG H pylori antibodies were determined using a modified ELISA technique.

Results—Of the 209 patients, 137 were infected with H pylori. The diagnostic value of systemic IgA and IgG H pylori antibodies was confirmed. Systemic IgA antibodies had a sensitivity of 76.6% (95% confidence interval 69.5–83.7) and a specificity of 94.4% (89.1–99.7). The sensitivity and specificity for systemic IgG antibodies were, respectively, 97.1% (94.3–99.9) and 98.6% (95.9–100). A moderate but clinically important correlation was found between local and systemic IgA and IgG. Mucosal IgA H pylori antibodies had a sensitivity of 98.5% (96–100) and a specificity of 91.7% (85.3–98.1), while for IgG these figures were, respectively, 88.3% (82.9–93.7) and 98.6% (95–100). As a diagnostic test mucosal IgA H pylori antibodies were comparable with culture and histology. Conclusion—Determination of local IgA and IgG H pylori antibody levels is a highly sensitive and specific test for the diagnosis of H pylori infection.

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Keywords: ELISA, Helicobacter pylori, IgA, IgG, serology.

Ten years after the successful culture of Helicobacter pylori, major progress has been made in the understanding of the pathogenic importance of this bacterium. H pylori causes chronic active gastritis, which is one of the most important pathogenic factors in peptic ulcer disease and may have a role in the pathogenesis of gastric carcinoma.

Several tests for diagnosing H pylori infection have been developed. These can be divided into direct tests, for which gastric biopsy specimens are required, and indirect tests which rely on serum or breath (13CO3, 14CO2) samples. Investigators are still polarised as to which test should be used for the diagnosis of H pylori infection. Proponents of indirect testing argue that upper gastrointestinal endoscopy is unnecessary and expensive, while for those in favour of direct tests the opportunity to detect macroscopic disease during endoscopy is paramount. In the present study we examined the diagnostic value of detecting local (direct) and systemic (indirect) IgA and IgG antibodies against H pylori using a modified in-house enzyme linked immunosorbent assay (ELISA) and compared this with culture and histology.

Methods

The study population comprised 209 consecutive patients (120 men and 89 women) with severe dyspeptic complaints who were referred for upper gastrointestinal endoscopy. Diagnostic tests for H pylori related gastritis were requested by the referring physician in all cases. Patients with an upper gastrointestinal malignancy and those taking antibiotics, bismuth preparations or omeprazole in the three months prior to the endoscopy were excluded.

After an overnight fast, an upper gastrointestinal endoscopy was performed in all patients and blood was obtained for serological tests. At endoscopy, biopsy specimens were taken from intact mucosa in the antrum 3–5 cm proximal to the pylorus. One biopsy specimen was set aside for culture of H pylori and was transported to the laboratory within two hours in a small sterile glass jar with a screw-cap in 2 ml sterile 0.9% NaCl. The biopsy specimen was cultured on blood agar (Blood Agar Base No 2, Oxoid CM 271, containing 5% sheep's blood) and Skirrow's medium, and cultures were incubated at 37°C in a nitrogen atmosphere containing 8% CO2 and 6% O2 for five days. The bacteria were identified as H pylori on the basis of their morphology, and oxidase, catalase, and urease production.

Four biopsy specimens, two from the anterior and two from the posterior wall, were obtained for histological assessment and measurement of local IgA and IgG H pylori antibodies. The
Mucosal *H pylori* antibodies two specimens for histological examination were immediately fixed in 5% buffered formalin, embedded in paraffin wax and cut into 4 μm thick serial sections, which were stained with haematoxylin and eosin. The histological detection of *Helicobacter*-like organisms was based on the identification, by an experienced pathologist, of micro-organisms with appropriate morphology, location, and staining characteristics in the mucosal biopsy specimens. Additional staining techniques (Giemsa, Whartin–Starry) were used where necessary.

Specific IgA and IgG antibodies against *H pylori* were measured in serum and in snap frozen antral biopsy specimens using a modified ELISA.

Antigen was prepared for the ELISA as follows: a mixture of six pooled *H pylori* strains (whole bacteria) was sonicated for six minutes. The suspension was adjusted to a protein concentration of 3 mg/ml. The optimum concentration of reagents was determined by checker board titration as described elsewhere.12 Each well of a flat bottom polystyrene microtitre plate (Dynatech Laboratories, Chandlilly, Virginia, USA; M129A) was coated with 100 μl antigen solution (10 μg suspension/ml carbonate buffer, pH 9-6, for IgA and the local IgA and IgG antibodies, and 1 μg suspension/ml for systemic IgG) and incubated overnight at room temperature. The plates were washed three times with phosphate buffered saline (PBS) (pH 7-5) containing 0-05% Tween 20.

Systemic specific IgA and IgG antibodies directed against *H pylori* were measured in serum, diluted 1 in 200 in PBS/Tween 20, by a modified ELISA technique using conjugates labelled with immunoperoxidase specific for human IgA and IgG. The following conditions were used to standardise antibody measurement: the mean (SD) values for absorbance of the standard reference sera were 0-5 (0-1) for IgA and 1-0 (0-1) for IgG. These values were used to correct the absorbance given by the serum samples under study.

The absorbance index (AI) was calculated from the mean of two readings of the optical density (OD) of the serum. The results were expressed as follows:

\[
AI = \frac{\text{Patient's OD} - \text{OD of blank reading}}{\text{Reference OD} - \text{OD of blank reading}}
\]

Intra- and interassay variabilities were determined as described in detail elsewhere.12 Local specific IgA and IgG antibodies directed against *H pylori* were also measured using a modified ELISA technique.

Biopsy samples were weighed and homogenised (Braun Potter S) in 300 μl PBS/Tween 20 and then adjusted to 1 mg/100 μl and stored at −20°C. The samples were then diluted 1 in 100 in PBS/Tween 20 and 100 μl was added to each well. Two reference samples (one positive and one negative) were also assayed. The plates were incubated at room temperature for 90 minutes and then washed three times in PBS/Tween 20. Diluted goat antihuman peroxidase (100 μl) was added to each well (Pasteur Institute, Paris, France; code no. 75051 diluted 1:1000 in PBS/Tween 20 for IgG and code no. 75041 diluted 1 in 2500 in PBS/Tween 20 for IgA). The plates were then incubated for 90 minutes at room temperature, washed and 100 μl substrate (0-40 mg/ml O-phenylenediaminedihydrochloride (Sigma, St Louis, Missouri, USA) containing 0-012% H2O2) was added. After 30 minutes the reaction was stopped with 50 μl 2-5 M H2SO4. The OD was measured at 492 nm on the Titertek Multiscan plate reader (Flow Laboratories, Irvine, Scotland, UK).

Before the data were analysed, the protein concentration (Lowry) of each biopsy homogenate was determined. To standardise the measurement of these antibodies, one positive serum (diluted 1 in 500) was tested more than 10 times in duplicate and the mean OD was used as a standard reference. The results were expressed as follows: A(1) = Mean OD reading (n = 2) of biopsy homogenate—mean OD of blank reading

\[
\times \frac{\text{mean OD reading (n = 2) of reference}}{\text{mean OD reading (n = 10) of reference}}
\]

\[
\times \frac{10 \text{ (percentage protein conc. of the homogenate)}}{\%
\]

Intra-assay variability for specific IgA *H pylori* antibodies was determined by measuring 17 homogenates (five with a low titre, six with a moderate titre and six with a high titre) six times in one plate, which resulted in a coefficient of variation of 6-9%. Intra-assay variability for specific IgG *H pylori* antibodies was also determined by measuring 17 homogenates (nine with a low titre, four with a moderate titre and four with a high titre) six times in one plate, which resulted in a coefficient of variation of 10-0%.

Interassay variability was determined by measuring specific IgA and IgG *H pylori* antibodies in the same biopsy homogenates (in duplicate) over five different days which resulted in coefficients of variation of 12-1% and 15-9%, respectively.

**Statistical analysis**

The significance of differences between test results in *H pylori* positive and negative patients was assessed using the Student's *t* test. To correct for multiplicity a p value <0-01 was chosen as the criterion for statistical significance. The significance of differences between the single diagnostic tests used was assessed using the χ² test.

**Results**

Of the 209 patients included in the study, 72 (mean age (SD) 47 (15) years) were *H pylori* negative while 137 (mean age (SD) 51 (13) years) were positive for *H pylori* on culture and/or histology. The diagnostic findings at upper gastrointestinal endoscopy are summarised in table 1.

In *H pylori* positive patients specific local (mean (SEM) 140·3 (5·2) v 2·8 (0·5), p<0·01 and systemic (mean (SEM) 81·6 (4·5) v 21·2
Table 1  Diagnoses at upper gastrointestinal endoscopy in H pylori negative and H pylori positive patients with severe dyspeptic complaints

<table>
<thead>
<tr>
<th>Patients</th>
<th>H pylori positive (n=137)</th>
<th>H pylori negative (n=72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis at endoscopy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No abnormalities</td>
<td>45 (32.8%)</td>
<td>27 (37.5%)</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>48 (35.0%)</td>
<td>13 (18.0%)</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>1 (0.7%)</td>
<td>2 (2.8%)</td>
</tr>
<tr>
<td>Reflux esophagitis</td>
<td>13 (9.5%)</td>
<td>17 (23.6%)</td>
</tr>
<tr>
<td>Other diagnosis</td>
<td>16 (11.7%)</td>
<td>9 (12.5%)</td>
</tr>
</tbody>
</table>

*Associated with non-steroidal anti-inflammatory drug use.

Table 2  Differences between local and systemic specific IgA and IgG H pylori antibodies in H pylori positive and H pylori negative patients. Results given as mean (SEM)

<table>
<thead>
<tr>
<th>Patients</th>
<th>H pylori antibodies (AI x 100)</th>
<th>H pylori positive (n=137)</th>
<th>H pylori negative (n=72)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local IgA</td>
<td>140 (5-2)</td>
<td>2 (8-5)</td>
<td></td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Systemic IgA</td>
<td>81 (6-4)</td>
<td>21 (3-3)</td>
<td></td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Local IgG</td>
<td>32 (2-3)</td>
<td>16 (0-3)</td>
<td></td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Systemic IgG</td>
<td>76 (6-2)</td>
<td>13 (0-9)</td>
<td></td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

*Student's t test.

Figure 1  Mucosal IgA H pylori antibodies versus systemic IgA H pylori antibodies in H pylori positive (●) and negative (○) patients. Line: regression (r=0.29; p<0.01) line in H pylori positive patients. The dotted lines indicate the cut off levels used in both assays.

Figure 2  Mucosal IgG H pylori antibodies versus systemic IgG H pylori antibodies in H pylori positive (●) and negative (○) patients. Line: regression line (r=0.52; p<0.01) in H pylori positive patients. The dotted lines indicate the cut off levels used in both assays.

Discussion

*Helicobacter pylori* induces a specific local[13-15] and systemic[16-17] immune response with the development of mucosa associated lymphoid tissue,[1,4,18-19] which is absent in normal stomach. The inflammatory changes in the gastric mucosa associated with this specific immune response are described as active chronic or chronic gastritis depending on the presence or absence of inflammation.
absence of leucocytes and lymphocytes infiltrating the gastric mucosa. These inflammatory changes are probably directly or indirectly responsible for functional derangements in the gastric physiology and mucosal defense mechanisms leading to peptic ulcer disease. Although the specific humoral immune response in most patients apparently does not lead to elimination of the bacterium from the gastric mucosa, it has successfully been used to diagnose H. pylori infection serologically using systemic circulating specific IgA and IgG H. pylori antibodies. Besides serological detection of H. pylori infection, H. pylori antibodies have also successfully been used for follow-up and in epidemiological studies. In the present study we confirmed the high sensitivity (97.1%) and specificity (98.6%) of systemic IgG H. pylori antibodies which correlated significantly with mucosal IgG antibodies. Although systemic IgA H. pylori antibodies were highly specific (94.4%), they were less sensitive for detecting H. pylori infection (76.6%), indicating that the predominant local production of IgA does not lead to high systemic IgA antibody levels in all patients (fig 1) rendering this test less suitable for diagnostic or epidemiological studies.

When compared with other more generally used non-invasive tests for diagnosing H. pylori infection only the urea breath test has a sensitivity and specificity comparable with that of the IgG ELISA. The applicability of the breath test is, however, hampered by its requirement for equipment and its cost. With a smaller number of commercial kits for IgA and IgG H. pylori antibodies becoming available it seems most likely that this non-invasive test will be more generally used. Direct tests can have low sensitivity and high inter- and intra-observer variation especially when the number of bacteria is reduced by suppressive treatment with bismuth preparations, antibiotics, or proton pump inhibitors. The direct test described herein may also facilitate follow up by demonstrating a decrease in the local production of H. pylori antibodies. For oral vaccines against H. pylori to be successful, the induction of a specific local IgA immune response is necessary. The level of local IgA H. pylori antibodies necessary for preventing reinfection (after treatment) or conveying protection (after oral vaccination) has yet to be established. In conclusion, determination of gastric mucosal IgA and IgG H. pylori antibody levels is highly sensitive and specific for the diagnosis of H. pylori infection.
Diagnosis of Helicobacter pylori infection by specific gastric mucosal IgA and IgG pylori antibodies.

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