Performance Characteristics of an Enzyme-Linked Immunosorbent Assay for Determining Salivary Immunoglobulin G Response to Helicobacter pylori

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We evaluated the salivary immunoglobulin G (IgG) immune response to Helicobacter pylori in 70 subjects by enzyme-linked immunosorbent assay (ELISA). Subjects with a positive H. pylori culture showed significantly higher titers of antibodies than subjects with no detectable H. pylori: the overall sensitivity and specificity of the test were 84 and 90%, respectively. The detection of salivary anti-H. pylori IgG antibodies may be considered as an alternative to serum IgG detection for ease of sample collection or when blood samples are not available in screening of patients with dyspepsia.

An association between colonization of the gastric mucosa with Helicobacter pylori and peptic ulcer, chronic gastritis, and gastric carcinoma has been clearly established (5, 15, 16, 19, 20). The high prevalence of H. pylori, arguably the most common cause of chronic bacterial infection in humans (infected 40 and 80% of individuals in developed and developing countries, respectively) (18, 19), demands the development of a noninvasive, sensitive, economic, and rapid technique of screening to detect infection. The current accepted diagnostic procedures for H. pylori infection include staining of histological samples, culturing of biopsy specimens, and the [13C]urea/[14C]urea breath test (2, 10, 11). The first two methods require invasive procedures and are therefore performed only for symptomatic subjects. The urea breath test, while not invasive, may be limited by its cost or the use of radiolabeled compounds (1). Although all strains of H. pylori cause some degree of persistent inflammation, the majority of people have no symptoms and a large number of infections may go undiagnosed (3, 4, 7, 17, 31, 33). Serological tests are available to evaluate total H. pylori-specific or H. pylori strain-specific antibodies (6, 12, 23, 26, 27, 32, 34), which could assist physicians in both diagnosis and follow-up of infected patients. Enzyme-linked immunosorbent assay (ELISA) is the most common commercial test whose performance is overall more accurate than that of latex agglutination or immunochromatography (27). Commercial Western blotting kits have recently been developed and are used to detect specific virulence markers (27, 28).

In this study, we investigated the salivary and serum anti-H. pylori immunoglobulin G (IgG) immune response in 50 dyspeptic patients and 20 healthy volunteers. As a preliminary definition of its clinical value, the performance of this assay was assessed against the histological, cultural, and biochemical detection of the bacterium. Multiple biopsy specimens, from the antrum and the body of the stomach, were obtained for histological, cultural, and biochemical examination. Five milliliters of venous blood and 5 ml of unstimulated saliva were obtained from all subjects. Specimens were stained with hematoxylin and eosin to evaluate the presence and severity of gastritis as well as with a modified Giemsa stain to identify H. pylori. Biopsy samples were cultured on pylori agar (bio-Merieux) within 1 to 2 h of endoscopy. The plates were incubated for up to 6 days in 7 to 8% carbon dioxide. Isolates were considered to be H. pylori if they grew as 0.5- to 1-mm translucent greyish colonies, were gram-negative curved or s-shaped rods, and were positive for oxidase, catalase, and urease. The urease test (CP test; Yamanouchi Pharma) was carried out according to the manufacturer’s instructions, and samples were examined for the presence of positive red color at 30 min and 24 h. Serum IgG against H. pylori antigens was detected by ELISA, using a commercially available kit (GAP test; BioRad). Samples were considered positive when antibody titers were >20 U/ml, as per the manufacturer’s guidelines. Evaluation of saliva IgG was performed by a modification of the above-described procedure. One hundred microliters of saliva was diluted 1:20 in phosphate-buffered saline supplemented with 1% (wt/vol) bovine serum albumin in duplicate wells. After a 1-h incubation at room temperature, a washing cycle with phosphate-buffered saline–0.05% Tween 20 was performed. One hundred microliters of peroxidase-conjugated anti-human IgG (supplied by the manufacturer) was added per well, and the plates were incubated for another hour at room temperature. A washing cycle was performed prior to the addition of 100 μl of freshly prepared substrate (H2O2) mixed with tetramethylbenzidine as a chromogen. The colorimetric reaction was then prolonged for 15 min at room temperature in the dark and terminated with the addition of 50 μl of 4 N H2SO4 per well. The absorbance values (optical density [OD]) at 450 nm were recorded with an automated reader. Antibody titers of the H. pylori-positive and -negative subjects were compared by Student’s t test; a P value of <0.05 indicated statistical significance. Sensitivity was defined as the ratio between true positives and true positives plus false negatives; specificity was defined as the ratio between true negatives and true negatives plus false positives. Accuracy was defined as the ratio between true positives plus true negatives and the total number of subjects. Positive predictive value (PPV) was defined as the ratio between true positives and true positives plus false pos-

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itives and negative predictive value (NPV) was defined as the ratio between true negatives and true negatives plus false negatives.

Gastric ulcer, duodenal ulcer, or gastric erosions were diagnosed endoscopically in 33 of 70 (47%) subjects (in 30 of 50 patients with dyspepsia and in 3 of 20 asymptomatic healthy volunteers). *H. pylori* positivity was confirmed by culture for 31 subjects, by histology for 34 subjects, and by urease testing for 33 subjects. One asymptomatic healthy subject was *H. pylori* positive. The degrees of concordance were 91% for culture and the urease test, 87% for culture and histology, and 84% between histology and the urease test. The concordance for all tests was 81%. Serum antibodies against *H. pylori* were detected in 91% (30 of 33) of patients with gastric lesions and in 100% (31 of 31) with positive *H. pylori* identification. The performance of the anti-*H. pylori* saliva IgG assay for *H. pylori*-positive patients was compared with that for *H. pylori*-negative patients as defined by the microbiological detection of *H. pylori* (Fig. 1a). *H. pylori*-positive patients showed significantly higher titers of anti-*H. pylori* IgG (mean OD ± standard deviation, 0.500 ± 0.392) than *H. pylori*-negative subjects (OD, 0.134 ± 0.06) (P < 0.05). True-positive rates (sensitivity) and false-positive rates (100% minus specificity) were calculated at different cutoff values and plotted to obtain a receiver operating characteristic curve (Fig. 1b). In this analysis, the point that encloses the largest area, i.e., the point that lies farthest to the “northwest” of the graph (OD, 0.21), represents the best compromise between sensitivity and specificity (22) and was chosen for our initial analysis. At this cutoff rate, the salivary IgG test was considered positive for 30 of 70 (42%) patients (Table 1). The test was positive for 26 of 31 *H. pylori*-positive patients (sensitivity, 84%) and 4 of 39 *H. pylori*-negative patients (specificity, 90%). Accuracy was 87%; the PPV and NPV were 86 and 88%, respectively (Table 2). A sensitivity of 100% with a narrow 95% confidence interval (89 to 100%) was obtained by employing a cutoff OD of 0.12 (31 of 31 positive tests for *H. pylori*-positive patients) (Fig. 1). The performance of the saliva test at this cutoff rate was identical to that of the serum IgG test (Table 1).

It is widely accepted that the serum anti-*H. pylori* IgG test is reliable for detecting *H. pylori* (12, 23, 27, 32). The diagnostic value and limitations of the GAP test ELISA have been addressed in several studies (8, 26, 27, 29). *H. pylori* antibodies can also be measured in salivary secretions, but the value of this procedure has not been established (9, 13, 14, 21, 24). In this study we have evaluated the performance of a test for the detection of salivary IgG against *H. pylori* and calculated its sensitivity and specificity at different cutoff values. The choice of cutoff line is an important parameter, as it affects the assay’s specificity and sensitivity in a reciprocal manner, with a major

### TABLE 1. Results of culture, IgG, urease tests, and histology

<table>
<thead>
<tr>
<th>Culture result</th>
<th>Salivary IgG</th>
<th>Serum IgG</th>
<th>Urease test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cutoff OD, 0.21</td>
<td>Cut-off OD, 0.12</td>
<td>Histology</td>
</tr>
<tr>
<td>+</td>
<td>26</td>
<td>5</td>
<td>31</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>35</td>
<td>19</td>
</tr>
</tbody>
</table>

### TABLE 2. Performance of IgG and urease tests and histology compared with culture

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Salivary IgG test</th>
<th>Serum IgG test</th>
<th>Histology</th>
<th>Urease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cutoff OD, 0.21</td>
<td>Cutoff OD, 0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>84 (66–95)</td>
<td>100 (89–100)</td>
<td>100 (89–100)</td>
<td>90 (74–98)</td>
</tr>
<tr>
<td>Specificity</td>
<td>90 (76–97)</td>
<td>51 (35–68)</td>
<td>51 (35–68)</td>
<td>85 (69–94)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>87 (77–94)</td>
<td>73 (61–83)</td>
<td>73 (61–83)</td>
<td>87 (77–94)</td>
</tr>
<tr>
<td>PPV</td>
<td>86 (69–96)</td>
<td>62 (47–75)</td>
<td>62 (47–75)</td>
<td>82 (65–93)</td>
</tr>
<tr>
<td>NPV</td>
<td>88 (73–96)</td>
<td>100 (83–100)</td>
<td>100 (83–100)</td>
<td>92 (78–98)</td>
</tr>
</tbody>
</table>

* Values are percentages. Data in parenthesis are 95% confidence intervals. Nonoverlapping confidence intervals are statistically different at a P < 0.05.
impact on the clinical decision-making process. A positive result at a cutoff level allowing for a high specificity (OD of 0.38 in our study) for a patient with ulcer and/or a positive urease test may justifiy the starting of therapy while awaiting the results of cultural and/or histological examination. When employed for population screening, on the other hand, a test requires a high sensitivity and, therefore, a lower cutoff. Its attendant low specificity may be an acceptable trade-off for certain clinical purposes. The high NPV at the cutoff of 0.12 could be of assistance in excluding patients from further invasive diagnostic procedures.

The sensitivity and specificity of a test depend upon certain characteristics of the population examined. Confounding factors, such as antibiotic therapy, may impact the performance of anti-\( H. \) pylori IgG assays. Five patients receiving anti-\( H. \) pylori therapy in the months prior to the study had positive salivary IgG tests but negative culture, histology, and urease results. This finding is consistent with the results of long-term serological surveillance studies (30) that showed that IgG antibody values were lower, but not yet within the normal range, 6 to 12 months after anti-\( H. \) pylori therapy. The possibility of “patchy” infections could also contribute to the low specificity of immunological tests compared to those of cultural or histological tests.

These results show that ELISA for detection of salivary anti-\( H. \) pylori IgG is a rapid, noninvasive, inexpensive test that may be considered as an alternative to the serum IgG test when blood samples are not available or in pediatric populations. While endoscopy and tissue biopsies remain irreplaceable for the definitive confirmation of the \( H. \) pylori status, the present study supports a role for the salivary IgG antibody response in screening patients with dyspepsia. Although certain ulcers and gastritis occur independently of \( H. \) pylori infection, a negative anti-\( H. \) pylori salivary IgG status may help in reducing the number of unnecessary endoscopies, especially in low-risk patients, such as subjects under 45 years of age (25).

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