Detection of Immunoglobulin G Antibodies to *Helicobacter pylori* in Urine by an Enzyme Immunoassay Method

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Urine and serum samples from 306 patients undergoing upper endoscopy were evaluated prospectively to determine the presence of immunoglobulin G (IgG) antibodies to *Helicobacter pylori* by an enzyme immunoassay method. Forty-nine selected urine specimens were also tested by Western blotting (immunoblotting). When compared with biopptic methods (culture, stain, urease testing), the sensitivity and specificity of the assay for urine IgG to *H. pylori* were 95.9 and 90%, respectively. Results of testing of serum and urine for IgG to *H. pylori* were concordant for 95% of samples. Western blot analysis revealed a highly variable antibody response to *H. pylori* antigens among patients. Detection of IgG antibody to *H. pylori* in urine is simple and reflects the presence or absence of *H. pylori* infection.

Evidence of a strong correlation between the presence of *H. pylori* on the gastric mucosa of patients with histologically confirmed gastritis, peptic ulcer disease, and gastric carcinoma stimulated the development of serological techniques for the detection of *Helicobacter pylori*. Many reports (6, 11) have shown that such serological techniques are simple and convenient for the detection of *H. pylori* in infected individuals. The advantage of serological methods over the more traditional biopptic methods for the identification of *H. pylori* is that the former methods are not complicated, time-consuming, expensive, or invasive.

The presence of antibodies to *H. pylori* in body fluids other than serum has also been reported. Rathbone et al. (12) demonstrated the presence of high levels of intragastric immunoglobulin A (IgA) antibodies in *H. pylori*-infected individuals. Czerniak et al. (4) studied salivary and gastric secretions for IgA and IgG antibodies against *H. pylori* and reported the presence of *H. pylori*-specific salivary IgG in infected individuals. Smith et al. (13) reported significant levels of salivary IgG and IgA to *H. pylori*, and Larsen et al. (7) used salivary IgG to assess the efficacy of antibiotic therapy for the eradication of *H. pylori*.

Specific IgG antibodies to *H. pylori* in urine have been measured in pilot studies (1–3, 11), and a good correlation between the levels of *H. pylori*-specific IgG in urine and sera of infected individuals has been shown. Antibody concentrations in urine, however, are far lower than those in serum. Because of their low concentration, a highly sensitive assay is required for detection of antibodies in urine. In addition to low antibody concentration, factors such as the patient’s renal function, urine constituents, and urine pH may influence antibody detection in urine. The development of an accurate, easy-to-use assay for the detection of *H. pylori* infections by using a fasting urine sample, however, seems advantageous. The primary purpose of the present study was to determine the utility of detecting IgG antibody to *H. pylori* in urine.

MATERIALS AND METHODS

**Patient population.** Urine and serum specimens were obtained from 306 patients (Table 1) attending the gastroenterology endoscopy unit at Los Angeles County and University of Southern California Medical Center for endoscopic evaluation of upper gastrointestinal symptoms. During endoscopy, paired biopsy specimens were obtained routinely from the gastric antrum and gastric corpus (as were biopsy specimens of any lesions). One biopsy specimen of each pair was used for culture by plating the specimen onto Skirrow’s Campylobacter medium (Anaerobic System, San Jose, Calif.) and incubating at 37°C under microaerophilic conditions (14). The second biopsy specimen was used for routine histological evaluation after staining with hematoxylin-eosin (H&E) (14). Additional biopsy specimens from the antrum were obtained for the CLO test (Delta West Limited, Bently, Australia), which detects urease activity by using phenol red as an indicator in an agar gel containing urea, and a liquid urease-catalase test, which consists of 1 ml of 2% urea-hydrogen peroxide solution containing bromothymol blue as an indicator, for the detection of urease and catalase. Both the CLO test and liquid urease-catalase test were interpreted after 20 min and 24 h. Specimens were obtained with patient consent under protocols approved by the Research Committee of Los Angeles County and the University of Southern California Medical Center.

**Preparation of antigens and ELISA method.** Briefly, the status of urine and serum IgG antibody to *H. pylori* was determined by using two strains of *H. pylori* (ATCC 43504 and ATCC 43579) obtained from American Type Culture Collection (ATCC; Rockville, Md.). Cultured bacteria were harvested in saline and were broken with glass beads. Specific antigens of *H. pylori* consisting of combined antigenic fractions with molecular sizes of 120, 84, 58 to 66, 30, and 14 kDa were used for Western blotting (immunoblotting) and PLYLORGEN enzyme-linked immunosorbent assay (ELISA) (HYCOR Biomedical Inc., Garden Grove, Calif.). Fresh postfasting undiluted urine specimens and serum samples diluted 1:250 were used in the ELISA. Serum samples with discordant positive and negative results compared with those obtained by biopptic methods were retested for IgG antibody to *H. pylori* by using Premier kits (Meridian Diagnostic Inc., Cincinnati, Ohio). Urinalysis was per-
formed on all urine specimens. Additionally, the pHs of nine urine specimens were adjusted to 7.0. This pH adjustment and the effect of freezing and thawing on 34 urine samples were examined. When urine was used as the specimen, the cutoff and incubation times were modified. The cutoff absorbance was determined by assaying urine from 50 patients with negative serology for *H. pylori* and whose biopsy specimens were negative for *H. pylori* by all bioptic methods (culture, H&E staining, and urease testing). Urine specimens with absorbance values which were above the absorbance values for negative urine specimens were considered positive. Each sample was assayed in duplicate at 450 nm.

Concentrated urine specimens from 46 selected patients who were infected with *H. pylori* (by all tests including culture) also were used in Western blotting. Fresh urine specimens were concentrated by ultrafiltration by using an Amicon pressure cell (Amicon, Danvers, Mass.) with a molecular mass cutoff of 10 to 30 kDa. The membrane was pretreated according to the manufacturer’s instructions and was stored in 10% ethanol at 4°C when not in use. Urine specimens (15 to 50 ml) were introduced into the ultrafiltration cell and were concentrated at 70 lb/in² with constant stirring. Each urine specimen was reduced to 4 ml and was washed with 10 ml of 100 mM phosphate buffer (pH 7.2). The concentration and washing steps were repeated once, yielding a final volume of urine (2 to 3 ml) which was mixed with an equal volume of glycerol, and the mixture was kept at −20°C until use.

**SDS-PAGE and Western blot analyses.** The protein components of *H. pylori* antigens were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and the resulting fractions were electrophoretically transferred to a nitrocellulose membrane (Bio-Rad, Richmond, Calif.). The membrane was blocked with bovine serum albumin, washed, and incubated with concentrated urine on a shaker set at midspeed for 6 h at room temperature. The membrane was washed and incubated again with anti-human IgG alkaline phosphatase conjugate (Jackson Immuno Research, West Grove, Pa.) and was subsequently stained with naphthylphosphate FAST-red substrate.

**RESULTS**

Selected characteristics of the 306 patients examined in the present study are listed in Table 1. Results of the various tests used in the present study for detection of *H. pylori* are presented in Table 2. *H. pylori* was detected by culture and/or H&E staining of endoscopically obtained biopsy specimens from any gastric site in 236 patients.

**Detection of antibody in serum and urine.** Of 236 patients who tested positive for *H. pylori* (by culture and/or H&E staining), 225 (95%) had serum IgG antibody to *H. pylori* and 11 (5%) did not have serum IgG antibody to *H. pylori* (Table 3). Of 70 patients who were negative for *H. pylori* (by culture and H&E staining), 58 (83%) had low IgG levels (negative) and 12 (17%) were positive. Of these 12 serologically positive patients, 8 patients were also urease (CLO and/or liquid urease) positive, and 11 of these 12 patients also tested positive for *H. pylori* with the Premier ELISA kit, which uses a high-molecular-mass cell-associated protein for the detection of IgG to *H. pylori*. Moreover, by using the Premier ELISA kit, 11 of the 11 patients with false-negative serological results also tested negative.

Of the 236 *H. pylori*-positive (by culture and/or H&E staining) patients, 227 were positive and 9 were negative for urine IgG antibody to *H. pylori*, and 16 of 70 *H. pylori*-negative patients (by culture and H&E staining) were positive for urine IgG antibody. Ten of these 16 patients also were urease (CLO and/or liquid urease) positive, and 8 of these 10 patients also were positive for serum IgG antibodies to *H. pylori*. In only 4 of 16 patients, serum and urine were positive for IgG antibodies to *H. pylori*, while all other tests were negative. Two remaining patients were positive for IgG antibodies to *H. pylori* in urine only. One of these two patients had more than 2,000 mg of protein per dl in urine.

To compare the presence of IgG antibodies to *H. pylori* in serum and urine, postfasting fresh urine specimens from all participating patients were tested in duplicate by ELISA for the presence of IgG antibodies to *H. pylori*. Results for 292 (95%) of 306 urine and serum specimens were in concordance. In 233 (80%) cases, both specimens were positive, and in 59 (20%) cases, both specimens were negative. Moreover, in 78% of the tested specimens, there was agreement between serum and urine IgG antibody titers. In 22% of the specimens which were negative for IgG antibodies to *H. pylori*, the ELISA was negative for IgG antibodies to *H. pylori*, while all other tests were negative. Two remaining patients were positive for IgG antibodies to *H. pylori* in urine only. One of these two patients had more than 2,000 mg of protein per dl in urine.

The diagnostic value of the ELISA for detection of IgG antibody in urine and serum is presented in Table 3. Compared with bioptic methods (culture, staining, or urease testing), the urine ELISA was 95.9% sensitive and 90% specific for the detection of *H. pylori* infection.

The effect of pH on the assay system was determined by adjusting the pH of nine selected specimens with pHs ranging from 5.0 to 7.5 and 7.0 and comparing the absorbances of pH-adjusted specimens with the original absorbances of the same specimens. Changes in urine pH had no significant effect on the absorbance value of the ELISA. Freezing of the urine specimen for 48 h at −20°C did not significantly affect the absorbance of the IgG antibody measurement; however, after long-term freezing at −20°C, false-positive or false-negative results occurred for 7 of 34 selected tested specimens. Microorganisms present in the urine specimens did not interfere with IgG antibody measurement in 10 selected urine specimens before and after filtration through a 0.2-μm-pore-size filter. Five patients with
very high urine IgG titers compared with the IgG serum

titers for the same patients had renal diseases indicated by a
high urine protein content (300 to >2,000 mg/dl). Four of five
patient specimens also had a high urinary glucose content.
One of these patients was positive only for urine IgG, indicating that patients with proteinuria may show false-
positive results.

Assay linearity. Assay linearity was obtained for serial
twofold dilutions of a positive urine specimen. The data
indicate a linear relationship between the measured \( A_{450} \)
and the log2 of dilution \( (r^2 = 0.991) \).

Western blotting. In order to demonstrate the presence
and complexity of \( H. pylori \)-specific IgG antibody in urine,
specimens from 46 \( H. pylori \)-positive patients were analyzed
by Western blotting. All 46 urine specimens positive for \( H. pylori \) by ELISA showed reactivity in the Western blot, but
the response to the \( H. pylori \) antigen fraction was not
consistent and varied from patient to patient. Many different
patterns were observed, with the most common (70%) anti-
gen fraction being 66 kDa. Urine specimens from patients
without evidence of \( H. pylori \) infection showed no reactivity
with \( H. pylori \) antigen fractions.

Urease tests. Of 236 patients positive for \( H. pylori \) (by
culture and/or H&E staining), 194 (82%) were CLO test

### TABLE 2. Summary of results of tests performed on all subjectsa

<table>
<thead>
<tr>
<th>No. of specimens</th>
<th>CLO test</th>
<th>Liquid urease test</th>
<th>Culture</th>
<th>H&amp;E staining</th>
<th>Serum IgG</th>
<th>Urine IgG</th>
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<tr>
<td>144</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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\( a \) Of 306 specimens tested, a total of 201 were positive by the CLO test, 232 were positive by the liquid urease test, 183 were positive by culture, 225 were positive by H&E staining, 237 were positive by serum IgG testing, and 243 were positive by urine IgG testing.

\( b \) Borderline serum samples were considered positive.

### TABLE 3. Performance of ELISA for detection of IgG antibodies in serum and urine versus endoscopic biopsy methods

<table>
<thead>
<tr>
<th>Test result</th>
<th>Culture and/or H&amp;E staining</th>
<th>ELISA</th>
<th>Any biopsy method( a )</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ELISA</td>
<td>( H. pylori ) positive (n = 236)</td>
<td>95.3</td>
<td>83.0( a )</td>
<td></td>
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<tr>
<td>Negative</td>
<td>( H. pylori ) negative (n = 70)</td>
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<tr>
<td></td>
<td>225 (A)</td>
<td></td>
<td>233 (A)</td>
<td></td>
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<td></td>
<td>11 (C)</td>
<td>58 (B)</td>
<td>14 (C)</td>
<td>55 (B)</td>
</tr>
<tr>
<td>Urine ELISA</td>
<td>Positive</td>
<td>96.1</td>
<td>77.1</td>
<td></td>
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<tr>
<td>Negative</td>
<td>227 (A)</td>
<td>16 (D)</td>
<td>237 (A)</td>
<td>6 (D)</td>
</tr>
<tr>
<td></td>
<td>9 (C)</td>
<td>54 (B)</td>
<td>10 (C)</td>
<td>53 (B)</td>
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\( a \) Culture, staining, or urease test.

\( b \) Sensitivity = \( A/(A + C) \).

\( c \) Specificity = \( B/(B + D) \).
positive and 221 (94%) were liquid urease test positive at 24 h. Sixty-five percent of the CLO tests and 73% of liquid urease tests were positive by 20 min.

**DISCUSSION**

It is now established that *H. pylori* is one of the most common bacterial pathogens in humans and is present in over 30% of individuals in the United States. The diagnosis of *H. pylori* infection usually involves invasive (endoscopic) methods or complex procedures such as the breath test (5, 8). Since infection by *H. pylori* induces the production of systemic antibodies, serological methods for the detection of *H. pylori*-specific IgG antibodies have been developed. Although it is usually not difficult to obtain blood, an easy-to-use urine test is simpler and less expensive. Urine specimens may be useful for screening both individuals and large populations for *H. pylori* infection. We determined the presence of anti-*H. pylori* IgG antibodies in the urine of 306 patients using a qualitative ELISA and found this to be a sensitive (95.9%) and specific (90%) test for the diagnosis of *H. pylori* infection when compared with biotic methods (culture, staining, or urease testing). Our immunoblot studies with concentrated urine specimens demonstrated that the antibody (IgG) response to *H. pylori* shows an inconsistent pattern from patient to patient. This finding confirms previous reports that immune responses to the *H. pylori* complex antigen demonstrate a remarkable complexity and diversity (9, 10). Each patient appeared to produce an apparently unique antibody pattern. A significant number of urine specimens tested did not react with the 30-kDa fraction, the protein fraction which is considered to be one of the main antigenic fractions of *H. pylori* (6). These results suggest that for detection of antibody to *H. pylori*, a complete mixture of *H. pylori* antigen fractions should be used.

Since the water content of urine, pH, microorganisms in the urine, and the patient’s renal status are factors which may influence the usefulness of urine as a specimen for antibody detection, we studied further the effects of those factors on assay performance. pH and the presence of microorganisms in urine had no significant effect on the absorbance value of the ELISA for antibody detection. We speculated that the long-term absence of urine may affect IgG antibody measurement because of the effect of urine components on antibody stability and/or, to some extent, protein precipitation because of freezing and thawing. The water content of urine is a factor which influences the concentration of antibody in urine. Our data show that performing a qualitative assay on postfasting urine was accurate (95% concordance between serum and urine samples), but we are unsure whether a random urine sample would be equally acceptable. The disagreement between titers in serum and urine for 22% of the specimens tested was mainly due to the water content of urine or the urine concentration. These findings show that detection of antibody in urine should be used in a qualitative assay system. Moreover, our findings show that antibody to *H. pylori* in urine can be measured in individuals of any race and that age and gender have no detectable influences on assay performance. In the present study, 10 (4%) of the patients tested false negative and 6 (10%) patients tested false positive for urine IgG when compared with biotic methods (culture, staining, or urease testing). Four of six patients with false-positive results also had a positive serological test for *H. pylori*. Of the remaining two patients, one had a high urine protein content. This observation possibly suggests that patients with significant proteinuria should be tested by alternative assay procedures. Additional studies in patients with proteinuria are needed to explore this issue further.

In summary, the primary purpose of the present study was to determine the feasibility and accuracy of detecting antibodies to *H. pylori* in urine specimens. We found that by using a urine specimen for detection of IgG antibodies to *H. pylori* in an ELISA, the sensitivity was 95.9% and the specificity was 90.0%. Since detection of antibodies in urine is simple, rapid, and inexpensive, we think that the use of urine as the specimen in an ELISA has great merit. Compared with current serological techniques, urine as a specimen may be a better choice for the measurement of anti- *H. pylori* IgG because urine is easier and cheaper to obtain.

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**REFERENCES**