Comparative evaluation of urine-based and other minimally invasive methods for the diagnosis of *Helicobacter pylori* infection

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**Introduction**

*H. pylori* infection is one of the most common infectious diseases in the world and has been found to be involved in upper digestive tract diseases, such as chronic gastritis, peptic ulcers, and gastric cancers. In addition, a potential role of *H. pylori* infection in several extra-digestive diseases, such as cardiovascular, respiratory, neurological, skin, and autoimmune diseases, has been reported. Therefore, there is an increased need for simple and noninvasive diagnostic methods for *H. pylori* infection in clinical practice and mass screening.

Initially, the rapid urease test, bacteriological culture, and gastric histological examination, which require endoscopic biopsy, were widely used for diagnosis. Serum antibody assay and the urea breath test (UBT), which do not require endoscopy, are now becoming more frequently used as diagnostic methods. Recently, new methods for the detection of anti-*H. pylori* antibody in urine and *H. pylori* antigen in stool have been developed. If sensitive screening for *H. pylori* infection were possible using urine as a sample, it would not only be more convenient in clinical practice but would also be very useful for mass screening. However, we do not yet have sufficient information on the usefulness of the newly developed noninvasive diagnostic methods that test urine.

To evaluate the usefulness of noninvasive urine-testing methods, we performed a study in healthy Japanese volunteers to compare the diagnostic accuracy of two tests for *H. pylori* antibody in urine with that of five serological tests and one test for antigen in stool samples, by using the UBT as the gold standard.

**Background.** Diagnostic methods have recently been developed for detecting anti-*Helicobacter pylori* antibody in urine and *H. pylori* antigen in stool samples. Our aim was to evaluate the usefulness of noninvasive urine-based methods for the diagnosis of *H. pylori* infection.

**Methods.** The study subjects were 100 asymptomatic Japanese volunteers. We investigated the diagnostic efficacy of various noninvasive diagnostic methods; five serological tests (Immunis anti-pylori, HM-CAP, EIAgén *Helicobacter pylori* IgG, Helico G, and GAP-IgG), one test for antigen in stool (HpSA enzyme immunoassay [EIA]), and two tests for antibody in urine (Urinelisa and Rapirun) by using the urea breath test (UBT) as the gold standard.

**Results.** Fifty subjects were diagnosed as positive for *H. pylori* infection by the UBT. The serological tests showed good sensitivity, specificity, and accuracy. The diagnostic values of the feces-based test (HpSA EIA) were lower than that of the serological tests. The sensitivities of the two urine-based methods in frozen urine samples were markedly lower than those of the other tests. However, the use of unfrozen samples markedly improved the diagnostic accuracy of these urine-based tests, which was then superior to that of the feces-based method.

**Conclusions.** This study clearly showed that urine-based tests were useful for the diagnosis of *H. pylori* infection. However, the use of frozen urine samples was not appropriate for the detection of anti-*H. pylori* antibody.

**Key words:** *Helicobacter pylori*, diagnosis, urine test, urea breath test, serological test, stool test

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Subjects and methods

Subjects

One hundred Japanese volunteers (50 men and 50 women) who had no dyspeptic symptoms and no history of gastrointestinal or hepatobiliary disease, and who were not taking any medication on a regular basis, were recruited for this study from among the staff of Shimane Medical University and their family members. Written informed consent was obtained from all subjects before they entered this study, which was carried out in accordance with the Declaration of Helsinki. It was confirmed that none of the subjects had undergone previous eradication therapy for *H. pylori*. None had been using any drugs, such as proton-pump inhibitors or antimicrobial medications, which might have affected the status of *H. pylori* infection, for at least 1 month.

Methods

The UBT was performed in all the subjects. Fasting blood, urine, and stool samples were collected within 7 days of the UBT. The examiners of each test were blind to the results of the other tests.

Urea breath test. The urea breath test (UBT) was performed as described previously, with minor modifications, after overnight fasting. 13C-Urea (100 mg) dissolved in distilled water was administered. Subjects were instructed to maintain left lateral recumbency for 5 min, followed by a sitting position for 15 min. Breath samples before and 20 min after administration of 13C-urea were collected after a mouthwash. The 13C-CO2 concentration in breath samples was measured by a 13C analyzer (Ubit-IR200; Otsuka Electronics, Osaka, Japan). Subjects whose δ13C values were lower than 2.5‰ were regarded as negative on the UBT test.

Serological tests. Fasting blood samples were immediately separated and stored at −20°C until the antibody titers were assayed. Five commercially available serum IgG antibody enzyme immunoassay (EIA) kits for *H. pylori* infection: Immunis anti-pylori (Institute of Immunology, Tokyo, Japan); HM-CAP (Epi New York, NY, USA); EIAgen *Helicobacter pylori* IgG (Biochem Immunosystems Italy, Bologna, Italy); Helico G (Axis-Shield Diagnostics, Dundee, UK); and GAP-IgG (Biomerica, Newport Beach, CA, USA) were employed in this study. Measurements were performed and cutoff values were set according to each manufacturer’s instructions in all tests. Subjects were divided into those with positive and negative results by Immunis anti-pylori and Helico G, and those with positive, negative, and intermediate results by HM-CAP, EIAgen, and GAP-IgG.

Detection of *H. pylori* antigen in stool. Stool samples were stored at −20°C until the assay was performed with a commercially available HpSA EIA kit (Meridian Diagnostics, Cincinnati, OH, USA). The test was performed according to the manufacturer’s instructions and the subjects were divided into those with positive, negative, and intermediate results.

Detection of antibody in urine. First, collected urine samples were stored at −20°C, without the addition of any preservatives, until the assay was performed with two commercially available kits: Urinelisa (Otsuka Pharmaceutical, Tokyo, Japan) and Rapirun (Otsuka Pharmaceutical). Urinelisa and Rapirun detect antibody for *H. pylori* in urine by enzyme-linked immunosorbent assay (ELISA) and immunochromatography, respectively. Both tests were performed with 0.5 ml urine, according to the manufacturers’ instructions.

Analysis. The results of the UBT were used as the gold standard in this study. The numbers of true and false positives and true and false negatives were calculated by comparing the individual results of each test with this final diagnosis. Sensitivity, specificity, and accuracy were calculated after the exclusion of subjects with intermediate or undiagnostic results.

Statistical analyses were performed by the Mann-Whitney U-test, χ² test, and McNemar test, with the aid of SPSS (6.1J version for Macintosh; SPSS, Chicago, IL, USA). Differences at two-tailed *P* < 0.05 were considered to be statistically significant.

Results

Fifty subjects were diagnosed as positive for *H. pylori* infection and 50 were negative by UBT. Of the positive subjects, 25 were men and 25, women, and of the negative subjects, 25 were men and 25, women (Table 1). Positive subjects were significantly older than negative ones.

Serological testing by Immunis anti-pylori and HM-CAP showed good sensitivity, specificity, and accuracy

<table>
<thead>
<tr>
<th>Table 1. Characteristics of subjects</th>
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<td><em>H. pylori</em>-positive</td>
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<td><em>H. pylori</em>-negative</td>
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*Significantly different from *H. pylori*-positive subjects (*P* < 0.05)