ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2000) 18: 63-67

Serodiagnosis of *Helicobacter pylori* Infection by Immunoblot Assay

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SUMMARY **We compared a noninvasive serological test using a commercial immunoblot assay (Helicoblot 2.0) to tissue-based methods [rapid urease test (CLO test), histology and culture] in eighty Thai patients undergoing upper endoscopy. A true positive test was defined as at least two of the biopsy-related tests being positive. The CLO test was the most accurate test with sensitivity and specificity as high as 100%, whereas histology and culture had sensitivity of 100% and 72.2%, respectively, and the specificity of 72.7% and 96%, respectively. The serological test had a high sensitivity (97.2%) but exhibited an unsatisfactory specificity (40.9%). We concluded that the rapid urease test using multiple gastric biopsies was the most appropriate method for diagnosing *H. pylori* status. The role of immunoblot assay as a serological screening test in our population remains doubtful, but it may identify patients who have been infected with certain strains of *H. pylori*.**

The role of *Helicobacter pylori* in gastroduodenal diseases has been elucidated during the past decade. It is now accepted as the main cause of chronic gastritis and is highly associated with peptic ulcer disease, as well as gastric cancer, including gastric mucosa-associated lymphoid tissue (MALT) lymphoma.[1-4] As the eradication of *H. pylori* has been shown to improve the outcome of peptic ulcer diseases in terms of recurrence and complications,[5] the accurate diagnosis of *H. pylori* infection, therefore, is clinically important.

Several invasive and non-invasive methods have been developed to diagnose *H. pylori* infection, however, no single test is accepted as the gold standard.[6] Each test has advantages and disadvantages which make it more or less appropriate for different situations. For example, the diagnosis of choice when endoscopy is performed is a rapid urease test (CLO test) due to its convenience.[7] Since the sensitivity of the test depends upon the number of bacteria present in the biopsy specimen, the test is unsuitable for determining the success of *H. pylori* eradication after treatment. Likewise, histologic examination is important, not only for the detection of *H. pylori* infection, but it also provides insight into gastric mucosal status.[8] The problems in using histology arise due to the results not only being dependent on the quality of the biopsy specimens but also the expertise of the pathologists.[9]

Culture is unquestionably the most specific and essential for research purposes, such as determining susceptibility *in vitro* or resistance to antibiotics.[10] However, it is time consuming, difficult to perform and has inadequate sensitivity to rule out infection, compared with other diagnostic techniques.[6,7,9] The serological test is a non-invasive method that is not influenced by sampling errors and

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is also useful in both diagnosis and screening of *H. pylori* infection. Nonetheless, the test is unable to differentiate past or present infection and is not accurate in assessing the success of eradication therapy.[11] Currently, various serological methods have been employed, such as bacterial agglutination test, immunochromatography, enzyme-linked immunosorbent assays (ELISAs) and immunoblotting techniques.[12] Among these, immunobloting assays seem to be more sensitive and specific than those of laboratory ELISA tests,[13] the most available commercial kits. From a recent study, the immunoblotting assays were considered to be as reliable as the urea breath tests in diagnosis of *H. pylori* infection.[14]

The aim of this study was to evaluate the diagnostic accuracy of an immunoblot assay (Helicoblot 2.0) in Thai patients with specific gastroduodenal diseases. We compared this test to the current tissue-based tests: endoscopy with gastric biopsy for CLO test, histology and culture.

MATERIALS AND METHODS

Population study

Eighty dyspeptic patients undergoing upper gastrointestinal endoscopy at Chulalongkorn University Hospital from October 1997 to May 1998 were included in the study. There were 52 male and 28 female patients whose ages ranged from 20 to 84 years (mean age 53.3 ± 14.9 years). Endoscopic findings were recorded and 6 biopsy specimens (3 each from the antrum and the body) were taken from each patient for histological examination, rapid urease test (CLOtest, Delta West, Bentley, Australia) and culture. Blood samples were taken after endoscopic examination and sera were separated by centrifugation and stored at –20°C until analyzed. Each patient gave informed consent to participate in the study.

Urease test on biopsy specimens

The CLO test was used for direct examination of urease activity in biopsy specimens. In principle, the test contains a urea substrate that is cleaved by urease into carbon dioxide and ammonia, causing a change in pH. The pH increase is detected by a phenol-red indicator. Two biopsy specimens from the antrum and the body were placed in the indicator, and the chromogenic reaction was read after 24 hours at room temperature.

Histologic examination of biopsy specimens

Two biopsy specimens from the antrum and the body were immediately fixed and transported in phosphate-buffered formalin (4%, pH 7.4). Sections of paraffin-embedded specimens were routinely stained with hematoxylin-eosin and a modified Giemsa staining.

Culture of *H. pylori*

Two biopsy specimens from the antrum and the body were transported in Stuart's medium and plated within 24 hours on 7% lysed, defibrinated horse blood agar plates. The plates were incubated under microaerobic conditions (5% O_2, 10% CO_2 and 85% N_2) at 37°C for 5 days. *H. pylori* forming small translucent colonies was identified as gram-negative, motile curved rods being urease-, oxidase-, and catalase-positive.

Immunoblotting assay

Immunoblot assay was determined by Helicoblot 2.0 (Genelabs Diagnostic, Singapore), as described previously.[15] In brief, the commercial kit used consisted of several serologically important antigens of *H. pylori*, including a 116 kDa (CagA) protein, a 89 kDa (VacA) protein, and the urease subunits. All buffers and reagents used were supplied with the kits. Membrane strips were incubated in wash buffer, after which a 1:100 dilution of sera in blocking buffer was added to each strip. Following a 1-hour incubation, the sera was aspirated and the strips were washed three times. After washing, alkaline phosphatase goat anti-human IgG conjugate diluted 1:1000 in blocking buffer was added to each strip and incubated for 1 hour. After washing, a substrate solution (5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium) was added to each strip, and the strip was incubated for 10-15 minutes. The substrate was then aspirated, following which the reaction was stopped with double distilled water. The strips were removed, dried and mounted on nonabsorbent paper. Serum-positive and negative controls were included with each batch of strips.

Statistical analysis

Sensitivity, specificity, positive and negative predictive values and diagnostic accuracy were calculated in accordance with standard methods.

RESULTS

It was found that 36 patients (45%) had non-ulcer dyspepsia (NUD), 20 patients (25%) had duodenal ulcer (DU), 22 patients (27.5%) had gastric ulcer (GU) and the remaining 2 patients (2.5%) had gastric cancer (GC).

Table 1 shows the results of the diagnosis of *H. pylori* infection by each method in this study. CLO test and histologic examinations were positive in 36 (45%) and 48 (60%) of these patients, whereas culture and immunoblot assay were positive in 28 (35%) and 61 (76.3%) cases, respectively. The *H. pylori* status was considered true positive when at least two of the biopsy-related tests (CLO test, histologic examinations and culture) were positive, while a true negative was considered when all these three tests were negative. Using these criteria, there were 36 (45%) true positive cases, 30 (37.5%) true negative cases and the remaining 14 (17.5%) cases were unclassified. The sensitivity, specificity, positive and negative predictive values and diagnostic accuracy of each test were calculated as shown in Table 2.

Table 1 Results of detection of *H. pylori* by each test

No. patients	CLO test	Histology	Culture	Immunoblot
26	+	+	+	+
9	+	+	-	+
1	+	+	-	-
2	-	-	+	+
4	-	+	-	+
8	-	+	-	-
20	-	-	-	+
10	-	-	-	-
Positive rate (%)	36	48	28	61

(+) positive results, (-) negative results

Table 2 Results of the sensitivity, specificity, positive and negative predictive values and diagnostic accuracy among the tests

	CLO test	Histology	Culture	Immunoblot
Sensitivity (%)	100	100	72	97
Specificity (%)	100	72	96	40
PPV (%)	100	75	93	57
NPV (%)	100	100	80	94
Accuracy (%)	100	85	85	66

PPV: positive predictive value, NPV: negative predictive value

In our study, the prevalence of *H. pylori* infection in GU patients by the above-mentioned criteria was 54.5% (12 of 22), slightly higher than in GC (50%, 1 of 2), NUD (47.2%, 17 of 36) and DU (40%, 8 of 20).

DISCUSSION

The prevalence of *H. pylori* infection using our criteria, which combined the results of CLO test, histology and culture was 45%. This prevalence is similar to that of other studies from Western countries where the prevalence was reported to be 42-68%.[16-18] However, it is somewhat lower than that found in previous reports from Thailand, with the prevalence accounting for 63-74%.[19-20] This discrepancy may be critically dependent on the different diagnostic criteria of *H. pylori* infection among studies. In fact, the definition of a 'gold standard' seems to vary from study to study. It is recommended that the reliable 'gold standard' should include two positive methods based on different principles to determine whether a patient is infected.[14]

The prevalence of *H. pylori* infection among those of DU and GU in the Thai population is lower than that reported from Western countries where the prevalence of *H. pylori*-associated DU and GU is as high as 90-95% and 70-80%, respectively.[19] Previous studies from Thailand showed that DU has the highest prevalence rate of *H. pylori* infection (66-77%), followed by GU and NUD with a prevalence of 52-55% and 44%, respectively.[20-21] The association of *H. pylori* with peptic ulcers in Thailand seems to be significantly lower than in Western countries. These data suggested that the etiology of peptic ulcers in Thailand may be other causes such as NSAIDs use, which has been reported to account for at least 25% of cases.[20]

Our study showed variation in the results obtained by different diagnostic methods. Among these, the CLO test is the only method to

identify *H. pylori* with a sensitivity and specificity over 90%. It has been suggested that the diagnostic yield of the test is partly dependent upon the location and number of biopsies obtained.[22] Therefore, using two gastric biopsies from the antrum and the body, as shown in this study, may be responsible for the increased sensitivity of the test. Apart from the accuracy shown in our study, its availability and rapidity, as well as its cost, make it most suitable as the test of first choice to detect *H. pylori*, particularly among patients undergoing upper endoscopy. Nonetheless, its sensitivity may be reduced in patients taking proton pump inhibitors (PPI), antibiotics or bismuth-based compounds. In such situations, alternative tests, for example, histology may be preferred.[23]

Among the non-invasive methods, serology is a valuable tool for seroepidemiological studies. However, its results depend upon several factors such as the types of tests, the *H. pylori* antigenic preparations and the population studied.[12] It is also agreed that the usefulness and practicality of the tests is dependent upon the background prevalence of *H. pylori* infection in a specific geographical area, as well.[23] For this reason, local validation of any blood test with another means of testing is essential. At present, the detection in sera of IgG antibodies against *H. pylori* by ELISA techniques is the most commercially available method. Commercial Western blotting kits have also been developed to detect the putative bacterial virulence factors including CagA and VacA, as well as other major antigens of *H. pylori*.[12]

In our study, using the immunoblot assay as the blood test a high sensitivity, over 90% was shown which was comparable with that of the CLO test and histology. Unfortunately, its specificity and positive predictive value were disappointing, accounting for only 40.9 and 57.4%, respectively. This result may lead to speculation that many patients have had their *H. pylori* infection eradicated in the past, either by spontaneous elimination[24] or by previous treatment with antibiotics for other reasons. This supports the hypothesis that *H. pylori* colonization is a dynamic process with an active phase of infection and subsequent elimination of the bacteria in a proportion of infected cases.[24]

Our data do not support the usefulness of the immunoblot assay as a screening test for determining *H. pylori* status.[12,13,25,26] A number of studies in Western countries have confirmed that infection with CagA-positive strains is associated with more severe gastritis and a higher prevalence of peptic ulceration and gastric carcinoma.[27-30] In contrast, studies in China and Japan demonstrate an equally high prevalence of CagA-positive strains in patients with peptic ulcer, gastric cancer, non-ulcer dyspepsia, and in control subjects.[31-32] These findings suggest possible genetic variations of bacterium strains in different geographical locations.

In conclusion, the immunoblot assay seems to be an inappropriate method of determining *H. pylori* infection in the Thai population due to its low specificity, since an accurate non-invasive test, such as the urea breath test, is still lacking in our area. The only reliable way to determine *H. pylori* status still requires an endoscopic examination. From our results, the CLO test exhibited the most appropriate method for the diagnosis of *H. pylori* infection with its rapidity, inexpensiveness and excellent diagnostic accuracy.

ACKNOWLEDGEMENTS

This work was supported by a Rajadapiseksompoj-China Medical Board research grant. We would like to thank Genelabs Diagnostics, Singapore, for providing the Helicoblot tests.

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