Evaluation of urine ELISA test for detecting *Helicobacter pylori* infection in Taiwan: A prospective study

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INTRODUCTION

Since the discovery of *Helicobacter pylori* (*H pylori*) by Marshall and Warren in 1983[1], overwhelming evidence has confirmed that *H pylori* infection plays a significant role in the development of chronic active gastritis, peptic ulcer, and gastric adenocarcinoma[2]. *H pylori* infection is very common throughout the world, occurring in 40-50% of the population in developed countries, 80-90% of the population in developing regions[3], and about 54.4% of the population in Taiwan[4].

A large number of methods have been developed to diagnose *H pylori* infection, including invasive and non-invasive tests. The former requires endoscopy exam for gastric mucosal biopsy. Because of the patchy nature of the infection, biopsy-based tests may suffer sampling errors[5]. There is an increasing interest in non-invasive tests, as they are not influenced by sampling error and can profitably replace endoscopy making the diagnosis and determining the management of some types of patients[6]. The urea breath test (UBT) is based on the carbon dioxide labeled with carbon-13 or carbon-14 in expired air to detect *H pylori* urease activity[7]. Serological tests are based on the detection of a specific anti-*H pylori* immunological response, mostly by IgG antibodies in patient's serum. Same as serum antibody testing, an enzyme immunoassay method (URINELISA test, Otsuka Pharmaceutical Co., Ltd, Japan) for detecting *H pylori* antibody in the urine has been marketed[8].

RESULTS: The sensitivity, specificity, positive predictive value, and negative predictive value of URINELISA are 91.7% (211/230), 90.8% (79/87), 96.3% (211/219), and 80.6% (79/98) respectively.

CONCLUSION: This URINELISA test is reliable, inexpensive and easy-to-use. The high diagnostic accuracy warrants the use of URINELISA as a first-line screening tool for diagnosis of *H pylori* infection in untreated patients.

Abstract

AIM: To evaluate the diagnostic accuracy and clinical utility of a new ELISA (URINELISA) test for detecting *Helicobacter pylori* (*H pylori*) antibody in the urine of Taiwanese population.

METHODS: In this prospective study, 317 consecutive dyspeptic patients (171 men, 146 women; mean age, 51.0 years) were included. They underwent gastroendoscopy for evaluation. Invasive tests, including culture, histology, and rapid urease test (RUT), and non-invasive 13C-urea breath test were preformed. At the same time, urine specimens were collected for URINELISA. The status of *H pylori* infection was considered as positive when either culture was positive, or when two of the other, RUT, histology or 13C-UBT, were positive.

RESULTS: The sensitivity, specificity, positive predictive value, and negative predictive value of URINELISA are 91.7% (211/230), 90.8% (79/87), 96.3% (211/219), and 80.6% (79/98) respectively.

CONCLUSION: This URINELISA test is reliable, inexpensive and easy-to-use. The high diagnostic accuracy warrants the use of URINELISA as a first-line screening tool for diagnosis of *H pylori* infection in untreated patients.

Key words: *H pylori*; URINELISA
treatment, chronic use of corticosteroids or immunosuppressant drugs, prior gastric surgery, presence of a bleeding peptic ulcer, severe concomitant disease, pregnancy, and lactation. Informed consent was obtained from each patient, and the study was performed in accordance with the Declaration of Helsinki.

**Study design**

All patients underwent gastroendoscopic examination, and gastric mucosal biopsies were performed. Non-invasive tests including URINELISA and 13C-urea breath test (13C-UBT) were also carried out. H pylori infection status was considered positive, when either culture was positive or when two of the following three tests, histology, rapid urease test (RUT), and 13C-UBT, were positive. Urine samples were collected on the same day after endoscopic examination. The endoscopic biopsy protocol is shown in detail as follows: two specimens from the antrum and body for culture, five specimens from the angle and both greater and lesser curvatures of the antrum and body for histology and four specimens, excluding angle, for RUT.

**Diagnostic tests for H pylori infection**

**Histology** One set of specimens was fixed with formalin and embedded in paraffin. Sections were then stained with hematoxylin and eosin (H&E).

**Rapid urease test** CLO-test (Delta West, Bentley, Australia) was selected for determination of the presence of urease in the biopsied gastric mucosa. The results of the CLO-test were interpreted as positive if the color of the gel changed from yellow to pink or red within 6 h at room temperature.

**Culture** Culture of H pylori was made by rubbing the specimen on the surface of a Campy-BAP agar plate [Brucella agar (Difco)+IsoVitalex (Gibco)+10% whole sheep blood], and then incubating it at 35 °C under microaerobic conditions (5% O2, 100 mL/L CO2 and 85% N2) for 4-5 d. The H pylori culture was considered positive if one or more colonies of gram-negative, oxidase (+), catalase (+), and urease (+) spiral or curved rods were present.

**13C-urea breath test (13C-UBT)** The 13C-urea was 100 mg 99% 13C-labeled urea produced by the Institute of Nuclear Energy Research (INER), Taiwan, and 100 mL of fresh milk was used as the test meal. This procedure has been modified since our previous study.[16] Patients were asked to fast at least 6 h beforehand, then a baseline sample was collected in duplicate by exhaling through a straw into a vacuum container tube 5 min after consuming the test meal. Five minutes later, the patients drank the urea solution prepared by dissolving 100 mg 13C-urea in 50 mL of sterile water. Immediately after 13C-urea consumption, subjects were asked to gargle rinsing the mouth to avoid detecting oral urease activity. The patient then rested on their sides for 15 min, changing sides every 5 min. Fifteen minutes after 13C-urea was ingested, a breathing sample was collected in duplicate as the method of collecting baseline samples. All samples were sent to INER, where a continuous-flow isotope ratio mass spectrometer (CF-IRMS, Europa Scientific Ltd, Crewe, UK) was used for analysis. Based on findings from our previous study, the cut-off value was 4.2/mL at 15 min after ingesting 13C-urea.

**URINELISA H pylori antibody test** All patients were asked to deliver a fresh urine specimen on the day of endoscopy. Urine samples were stored at 4 °C before analysis. Urinary antibodies to H pylori were determined by URINELISA H pylori antibody test (Otsuka Pharmaceutical Co., Ltd, Japan). This kit is an enzyme-linked immunosorbent assay (ELISA) kit using a 96-well microplate, and, as a solid phase of an antigen, a protein extracted from an H pylori strain isolated from a Japanese patient with gastritis is used. The assay procedure was performed according to the manufacturer’s recommendations. To each well of the solid-phase microplate, 25 μL each of a buffer solution and 100 μL each of the urine specimen, the negative controls or positive controls were added in turn. After a reaction at 37 °C for 1 h, the plate was washed. One hundred microliters of an enzyme-labeled antibody solution was added to each of the wells subjected to the reaction at 37 °C for 1 h, and then washed. One hundred microliters of a substrate solution was then added. After the reaction was conducted for 15 min, 100 μL of a reaction stopping solution was added to each well. Absorbance was determined at a wavelength of 450 nm by an ELISA reader. Two positive controls and three negative controls were measured simultaneously. The cut-off value for urine-based ELISA was determined as the value calculated by the following formula: (mean absorbance of two positive controls)/8.5+ (mean absorbance of three negative controls). The cut-off value was assessed as negative, and absorbance greater than the cut-off value was assessed as positive.

**RESULTS**

Of 317 patients, 230 (72.6%) were H pylori-infected and 87 (27.4%) were uninfected. Of the infected patients, 75 (23.7%) had non-ulcer dyspepsia, 224 (70.7%) had duodenal and gastric ulcers, and 18 (5.7%) had gastric adenocarcinoma. URINELISA was positive in 219 cases (8 of which were false positives) and negative in 98 (19 of which were false negatives). A summary of the diagnostic efficacy of individual tests is shown in Table 1. The urine test showed

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Table 1 Summary of diagnostic efficacy of all tests used in this study

| Test                | URINELISA (%) | 13C-UBT (%) | RUT (%) | Culture (%) | Histology (%) |
|---------------------|---------------|-------------|---------|-------------|---------------|
| Sensitivity         | 91.7 (88.7–94.8) | 96.7 (94.7–98.7) | 94.4 (91.9–96.9) | 55.6 (50.1–61.1) | 86.7 (83–90.4) |
| Specificity         | 90.8 (87.6–94.0) | 95.2 (92.8–97.6) | 95.2 (92.8–97.6) | 100 (100)     | 79.7 (75.3–84.1) |
| PPV                 | 96.4 (94.3–98.5) | 96.7 (94.7–98.7) | 96.6 (94.6–98.6) | 100 (100)     | 85.6 (81.7–89.5) |
| NPV                 | 80.6 (76.2–85.0) | 95.2 (92.8–97.6) | 92.3 (89.4–95.2) | 61.2 (55.8–66.6) | 80.6 (76.2–85) |

Results are expressed by percentages, with a 95% CI.
sensitivity of 91.7%, specificity of 80.8%, positive predictive value of 96.4% and negative predictive value of 80.6%. That is, the diagnostic efficacy of URINELISA was similar to 14C-UBT and RUT.

DISCUSSION

Since the early 1980s, when _H. pylori_ was first discovered, significant progress has been made in identifying this infection, especially with respect to non-invasive techniques. The UBT, first described by Graham _et al._, in 1987 [27], is presently considered as the most effective non-invasive test for _H. pylori_ [28]. Based on the direct identification of a bacterial enzyme activity (urease), the UBT detects an active, ongoing infection and is highly accurate even shortly after therapy [29]. However, the availability of 14C-UBT is limited by the strict regulations governing the use, handling, and storage of radioactive isotopes, which in practice precludes use outside hospitals and the testing of children and women of childbearing age. 13C on the other hand is a safe isotope that can be used for breath testing, but it is expensive (about $250-350) when compared with other non-invasive tests (about $20-100) [30], and does require the use of a CF-IRMS, thereby limiting widespread applicability. Furthermore, UBT for detection of _H. pylori_ is less effective in patients who have had a prior gastrectomy [21] and in those with severe atrophic gastritis [22]. In addition, equivocal or false-negative UBT results often occur in patients taking acid-suppression therapy [23]. Moreover, in most of the approved UBT protocols, demands are made on patients’ time and cooperation (i.e., fasting before the test, the necessity of an office/laboratory visit, consumption of a test meal, and collection of a second breath sample after a time lag). Therefore, it is not easy to perform a UBT test on patients who cannot cooperate, such as children, psychological patients and unconscious patients.

Serology has clear advantages over breath tests, both in terms of cost and in being a non-invasive screening strategy, and it is the most important tool for epidemiological studies [26]. However, old-fashioned serological tests have suffered from cross-reactivity with _Campylobacter_ species [28], resulting in a high rate of false positives. The reported sensitivity of serological tests is approximately 98-100%, but specificity varies from 26% to 96.4% [29]. Results of these assays may be due to the reference method used to confirm _H. pylori_ status, the source of the antigen on which the assay is based, and the reference population studies [29]. Furthermore, the specificity of most serologic tests decreases by approximately 10%, when sera from subjects more than 45 years are examined [30].

Recently, urine-based antibody tests have been developed for the screening of infectious diseases, including _H. pylori_ [21]. The benefits of using urine as the specimen are as follows: (1) Urine can be obtained easily, and its collection requires little skill. (2) Urine does not require centrifugation. (3) The cost of using urine as a sample is much lower than that of serum. (4) Urine tests can be simply formatted for use in doctors’ offices or even for home testing. (5) Using urine provides a simpler alternative sample for epidemiological and screening studies. Laboratory-based urine testing using ELISA technology to detect IgG antibody is inexpensive (under $20), non-invasive, and well suited to primary care practice. Urine-based testing also combines the advantages of simplicity, cost-effectiveness and applicability under circumstances in which serological testing is largely impracticable. It has been reported that antibodies in urine are stable for 55 days at room temperature, and for one year at 2-8 °C [31]. Katsuragi _et al._, also confirmed that _H. pylori_ antibodies in urine were viable for at least 60 d at 4 °C and 25 °C, and for at least 3 d at 37 °C and 45 °C. In contrast, stool samples must be kept at 4 °C within 12 h after defecation, or diagnostic efficacy decreases.

Two studies have been performed in different populations with similar diagnostic efficacy with our results reported [32,33]. These studies show that URINELISA has a high degree of consistency in detecting _H. pylori_ infection in both Asian and European populations. Therefore, URINELISA is suitable for mass screening of _H. pylori_ infection. Such consistency has not been observed in stool antigen tests. In our own experience [33], and based on other reports made of an Asian population [34,35], the diagnostic efficacy of Premier Platinum HpSA® has been less consistent in an Asian population. However, there is a new stool antigen test, ImmunoCard HpSA STAT®, which seems to have higher diagnostic accuracy in Asian population [36]. In conclusion, URINELISA test is reliable, inexpensive and easy-to-use, and its high degree of diagnostic accuracy warrants its use as a first-line screening tool for the diagnosis of _H. pylori_ infection in untreated patients.

REFERENCES

1. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983; 1: 1273-1275
2. International Agency for Research on Cancer WHO. Infection with _Helicobacter pylori_. In: Schistosomes, liver flukes and _Helicobacter pylori_. Lyon: IARC 1994: 177-202
3. Vaira D, Miglioli M, Mule P, Holton J, Menegatti M, Vergura M, Biasco G, Conte R, Logan RP, Barbara L. Prevalence of peptic ulcer in _Helicobacter pylori_ positive blood donors. Gut 1994; 35: 309-312
4. Lin JT, Wang JT, Wang TH, Wu MS, Lee TK, Chen CJ. _Helicobacter pylori_ infection in a randomly selected population, healthy volunteers, and patients with gastric ulcer and gastric adenocarcinoma. A seroprevalence study in Taiwan. Sand J Gastroenterol 1993; 28: 1067-1072
5. van Zweit AA, Thijs JC, Roosendaal R, Kuipers EJ, Pen A, de Graaff J. Practical diagnosis of _Helicobacter pylori_ infection. Eur J Gastroenterol Hepatol 1996; 8: 501-507
6. Current European concepts in the management of _Helicobacter pylori_ infection. The Maastricht Consensus Report. European _Helicobacter pylori_ Study Group. Gut 1997; 41: 8-13
7. Logan RPH. The 13C urea breath test: In Lee A, Megraud F, Eds. _Helicobacter pylori_: technique for clinical diagnosis and basic research. WB Saunders 1996: 74-81
8. Katsuragi K, Noda A, Tachikawa T, Azuma A, Mukai F, Murakami K, Fujioka T, Kato M, Asaka M. Highly sensitive urine-based enzyme-linked immunosorbent assay for detection of antibody to _Helicobacter pylori_. _Helicobacter_ 1998; 3: 289-295
9. Miwa H, Hirose M, Kikuchi S, Terai T, Iwasaki R, Kobayashi O, Takei Y, Ogihara T, Sato N. How useful is the detection kit for antibody to _Helicobacter pylori_ in urine (URINELISA) in clinical practice? Am J Gastroenterol 1999; 94: 3460-3463
10. Yang JG, Wang TH, Wang HJ, Kuo CH, Wang JT, Wang WC. Genetic analysis of the cytotoxin-associated gene and the
vacuolating toxin gene in Helicobacter pylori strains isolated from Taiwanese patients. Am J Gastroenterol 1997; 92: 1316-1321

Shyu RY, Jhang SY, Lai CH, Hsu CT, Young TH, Yeh MY. High frequency of cytotoxin-associated gene A in Helicobacter pylori isolated from asymptomatic subjects and peptic ulcer patients in Taiwan. J Clin Gastroenterol 1998; 27: 54-59

Maeda S, Ogura K, Yoshida H, Kanai F, Ikenoue T, Kato N, Shiratori Y, Omata M. Major virulence factors, VacA and CagA, are commonly positive in Helicobacter pylori isolates in Japan. Gut 1998; 42: 338-343

Miwa H, Kikuchi S, Ohtaka K, Kobayashi O, Ogihara A, Hojo M, Nagahara A, Sato N. Insufficient diagnostic accuracy of imported serological kits for Helicobacter pylori infection in Japanese population. Diagn Microbiol Infect Dis 2000; 36: 95-99

Leung WK, Ng EK, Chan FK, Chung SC, Sung JJ. Evaluation of three commercial enzyme-linked immunosorbent assay kits for diagnosis of Helicobacter pylori in Chinese patients. Diagn Microbiol Infect Dis 1999; 34: 13-17

Wang WM, Lee SC, Ding HJ, Jan CM, Chen LT, Wu DC, Liu CS, Peng CF, Chen YW, Huang YF, Chen CY. Quantification of Helicobacter pylori infection: Simple and rapid 13C-urea breath test in Taiwan. J Gastroenterol 1999; 33: 330-335

Kato M, Asaka M, Saito M, Sekine H, Ohara S, Toyota T, Akamatsu T, Kaneko T, Kiyosawa K, Nishizawa O, Kumagai T, Katsuyama T, Abe M, Kosaka M, Hariya S, Minami K, Sanai Y, Sawamura M, Tachikawa T. Clinical usefulness of urine-based enzyme-linked immunosorbent assay for detection of antibody to Helicobacter pylori: a collaborative study in nine medical institutions in Japan. Helicobacter 2000; 5: 109-119

Graham DY, Klein PD, Evans DJ, Evans DG, Alpert LC, Opekun AR, Boutton TW. Campylobacter pylori detected during treatment with omeprazole. Am J Gastroenterol 1995; 90: 1174-1177

Atherton JC, Spiller RC. The urea breath test for Helicobacter pylori. Gut 1994; 35: 723-725

Vaira D, Malfertheiner P, Megraud F, Axon AT, Deltenre M, Gasbarrini G, O’Morain C, Pajares Garcia JM, Quina M, Tytgat GN. Noninvasive antigen-based assay for assessing Helicobacter pylori eradication: a European multicenter study. The European Helicobacter pylori Hp5A Study Group. Am J Gastroenterol 2000; 95: 925-929

Smoot DT, Cutler AF. Helicobacter pylori. Diagnostic tests. Gastroenterology and Endoscopy News. McMahon Publishing Group, New York 1997; 48: 28

Chen X, Haruma K, Kamada T, Mihara M, Komoto K, Yoshihara M, Sumii K, Kajiyama G. Factors that affect results of the 13C urea breath test in Japanese patients. Helicobacter 2000; 5: 98-103

Logan RP, Walker MM, Misiewicz JJ, Gummett PA, Karim QN, Baron JH. Changes in the intragastric distribution of Helicobacter pylori during treatment with omeprazole. Gut 1995; 36: 12-16

Graham DY, Genta R, Evans DG, Reddy R, Clarridge JE, Olson CA, Edmonds AL, Siepman N. Helicobacter pylori does not migrate from the antrum to the corpus in response to omeprazole. Am J Gastroenterol 1996; 91: 2120-2124

Chey WD, Spybrook M, Carpenter S, Nostrant TT, Elta GH, Scheiman JM. Prolonged effect of omeprazole on the 14C-urea breath test. Am J Gastroenterol 1996; 91: 89-92

Chey WD, Woods M, Scheiman JM, Nostrant TT, DelValle JE. Lansoprazole and ranitidine affect the accuracy of the 14C-urea breath test by a pH-dependent mechanism. Am J Gastroenterol 1997; 92: 446-450

Feldman RA, Evans SJ. Accuracy of diagnostic methods used for epidemiological studies of Helicobacter pylori. Aliment Pharmacol Ther 1995; 9(Suppl 2): 21-31

Yamamoto I, Fukuda Y, Mizuta T, Fukada M, Nishigami T, Shimoyama T. Serum anti-Helicobacter pylori antibodies and gastritis. J Clin Gastroenterol 1995; 21(Suppl 1): S164-168

Marchildon PA, Ciota LM, Zamanian FZ, Peacock JS, Graham DY. Evaluation of three commercial enzyme immunoassays compared with the 13C urea breath test for detection of Helicobacter pylori infection. J Clin Microbiol 1996; 34: 1147-1152

Schembri MA, Lin SK, Lambert JR. Comparison of commercial diagnostic tests for Helicobacter pylori antibodies. J Clin Microbiol 1993; 31: 2621-2624

Alemohammad MM, Foley TJ, Cohen H. Detection of immunoglobulin G antibodies to Helicobacter pylori in urine by an enzyme immunoassay method. J Clin Microbiol 1993; 31: 2174-2177

Kostolansky F, Li JJ, Friedman-Kien AE. Detection of false antibodies to HIV-1 in urine. AIDS 1997; 11: 1533-1532

Bravo LE, Nearle JL, Campo C, Mera R, Correa P. Effects of acid suppression and bismuth medications on the performance of diagnostic tests for Helicobacter pylori infection. Am J Gastroenterol 1999; 94: 2380-2383

Leodolter A, Vaira D, Bazzoli F, Schutzke K, Hirschl A, Megraud F, Malfertheiner P. European multicentre validation trial of two new non-invasive tests for the detection of Helicobacter pylori antibodies: urine-based ELISA and rapid urine test. Aliment Pharmacol Ther 2003; 18: 927-931

Adachi K, Kawamura A, Ono M, Masuzaki K, Takashima T, Yuki M, Fujishiro H, Ishihara S, Kinoshita Y. Comparative evaluation of urine-based and other minimally invasive methods for the diagnosis of Helicobacter pylori infection. J Gastroenterol 2002; 37: 703-708

Yu FJ, Wu DC, Kuo CH, Lu CY, Su YC, Lee YC, Lin SR, Liu CS, Jan CM, Wang WM. Diagnosis of Helicobacter pylori infection by stool antigen test in southern Taiwan. Kaohsiung J Med Sci 2001; 17: 344-350

Wong BC, Xiao HX, Cheung HK, Ng FH, Wong SY, Chow KC, Lin SK, Yin Y, Wong WM, Yuen MF, Lam SK. Evaluation of two stool antigen tests for the detection of Helicobacter pylori infection in the Chinese population. J Gastroenterol Hepatol 2003; 18: 26-31

Wu IC, Ke HL, Lo YC, Yang YC, Chuang CH, Yu FJ, Lee YC, Jan CM, Wang WM, Wu DC. Evaluation of a newly developed office-based stool test for detecting Helicobacter pylori: an extensive pilot study. Hepatogastroenterology 2003; 50: 1761-1765

Li YH, Guo H, Zhang PB, Zhao XY, Da SP. Clinical value of Helicobacter pylori stool antigen test, ImmunoCard STAT Hp5A, for detecting H pylori infection. World J Gastroenterol 2004; 10: 913-914

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