Usefulness of the *Helicobacter pylori* stool antigen test for detection *Helicobacter pylori* infection

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Abstract

Several diagnostic tests are available for evaluating *Helicobacter pylori* (*H.pylori*) infection : histological examination, culture of gastric biopsy specimens, rapid urease test, urea breath test and serology. In this study, we assessed the reliability of a newly developed enzyme immunoassay HpSA (*H. pylori* Stool Antigen) kit for detecting *H. pylori* antigen in stool. Eighty-five patients (50 males, 35 females ; mean age 41.6 ± 9.8 years) with dyspeptic symptoms who were examined by upper gastrointestinal endoscopy. The patients with a history of previous treatment with proton pump inhibitors, bismuth compounds or antibiotics were excluded. During the endoscopic examination biopsies were taken from antrum and corpus for rapid urease test and histological examination. Stool specimens were submitted to the laboratory and HpSA test was performed. *H. pylori* was considered in condition with rapid urease test and histopathological examination for *H. pylori* positive. Fourty-six of 85 patients were positive and remaining 39 patients were negative for *H. pylori* with the rapid urease test and pathologic evaluation. When 0.160 was adopted as the cut-off value, in accordance with the manufacturer's recommendations ; stool antigen has been detected in 45 of the 46 *H.pylori* positive patients. The sensitivity and specificity of HpSA test were 97.8%, 94.9% respectively.

These results indicate that HpSA is a highly reliable diagnostic method for *H. pylori* infection. (Acta gastroenterol. belg., **2002**, 65, **74-76**).

Key words : *H. pylori* stool antigen, urease test, histopathological examination.

Introduction

Helicobacter pylori (*H.pylori*) causes chronic gastritis and also has a role in development of gastric and duodenal ulcer. In many report showed that *H. pylori* is involved in gastric carcinogenesis and is regarded as a possible important factor in at least a subset of patients with functional dispepsi (1). *H. pylori* is now being actively investigated for a possible involvement in various nongastroenterological condition such as impaired growth, coronary heart disease, headache, Reynaud's phenomen, diabetes and gallstones disease (2). *H. pylori* causes chronic gastric infection, which is usually lifelong lasting and many epidemiological studies have shown that this is probably one of the most common bacterial infection throughout the world. Possibly it is effecting 50% of the population in developed countries and up to 80-90% of the population in effecting regions (3). Invasive and noninvasive diagnostic tests are available for diagnosis of *H. pylori* infection, including histopathological examination and culture of gastric biopsy specimens, rapid urease test, urea breath test (UBT) and serology (4-6). Each of the available technique has advantages as well as disadvantages, and it is now clear that the discussion over the different diagnostic methods cannot be oversimplified by thinking just in term of "which is the best diagnostic tool ?" (7). In this study, we tested non-invasive method in the diagnosis of *H. pylori* infection by using stool samples. This method is a new direct Enzyme Immunoassay (HpSA) which detected *H. pylori* infection. US Food and Drugs Administration (FDA) had given approval for the HpSA test for two conditions : 1) Diagnosis of *H. pylori* infection in adult symptomatic patients, and 2) monitoring response to therapy in adult patients. The test utilises polyclonal anti-*H. pylori* capture antibody absorbed to microwells, the colour of disc develops in the presence of bound *H. pylori* antigens. Finally stop solution is added and the results are read spectrophotometrically (i.e. *H. pylori* antigens are present in the stool samples) (8).

Material and methods

This study included 85 patients (50 males, 35 females ; mean age 41.6 ± 9.8 years) complaining of recent onset of dyspeptic symptoms, who were submitted to upper gastrointestinal endoscopy between February '2000 and June '2001. Exclusion criteria were previous treatment with proton pump inhibitors, bismuth compounds or antibiotics and pregnancy, lactation, severe systemic illness, manifest clotting disorders or use of anticoagulants. During the endoscopic examination two biopsies were taken from antrum and corpus for rapid urease test (CLOtest, Delta West Ltd Australia) and histopathological examination, and stool specimens were submitted to the laboratory to be stored at –20ºC degree until the HpSA test (Meridian Diagnostics Inc. USA) was performed. *H. pylori* was accepted to be present, if histopathological examination and rapid urease test were positive.

Antigen detection in stool : Totally 85 stool samples were diluted 1:5 in sample diluent, and 50 ml was added to each antibody-coated microwell. One drop of enzyme

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Table 1. — **Histopathology, rapid urease test and HpSA findings**

HpSA	Histopathology and rapid urease test		Total
	Positive	Negative	
HpSA (positive)	45	2	47
HpSA (negative)	1	37	38
Total	46	39	85

(Histopathology and rapid urease test were in accordance with 46 *H. pylori* positive and in 39 *H. pylori* negative patients).

conjugate was added to the microwells which were then incubated at room temperature for 1 hour, and washed five times. Two drops of substrate were added to each well, followed by a 10 min incubation period at room temperature. One drops stop solution was added to each well, and the plates were read spectrophotometrically at Δ450 nm. A Δ450 of 0.160 was considered positive as the cut-off value, in accordance with the manufacturer's recommendations.

Results

In endoscopic examination, 56 patients showed normal mucosa, 11 had active or scarred gastric or duodenal ulcers and 18 gastric or duodenal erosions. Of the 85 patients, 46 had positive (23 without endoscopic lesions, 10 with active or scarred peptic ulcer, 13 with erosions) and 39 had negative (28 without endoscopic lesions, none with active or scarred peptic ulcer, 11 with erosions) test for *H. pylori* by both rapid ürease test and pathological evaluation. The HpSA results are given in Table 1. Stool antigen has been detected in 45 of the 46 *H. pylori* positive patients. The sensitivity and specificity was 97.8% and 94.9% respectively.

Discussion

At present, several reliable methods for detecting *H. pylori* infection are available. Each has unique features, advantages, but none is suitable for all situations (9). However since invasive methods require endoscopy, they are neither suitable for mass screening nor applicable to patients. Furthermore false negative results caused by sampling error cannot be avoided, especially after eradication therapy. A combination of tests is commonly needed, depending on the clinical indication. Invasive techniques (histopathology, culture and rapid urease tests) are expensive and mostly advisable when the endoscopic examination can yield important clinical information about lesions (10).

In young dyspeptic patients, in whom endoscopy is not mandatory, rapid, inexpensive, easy–to-use and reliable techniques are needed to detect *H. pylori*. The two main non-invasive tools for the diagnosis of *H. pylori* infection are the Urea Breath Test (UBT) and serological tests. The breath test is easy to perform and generally has excellent sensitivity and specificity, very close to 100% (11). It can detect eradication of *H. pylori* four weeks after antibiotic therapy, when serological tests still give a positive result. However, the high, though falling, costs and complexity of equipment have limited the spread of this test (12).

Serological methods are non-invasive, relatively speedy, simple to perform and less expensive than the breath test or invasive tests (13,14), but as blood must be obtained, this can sometimes be a problem, mostly in children. Furthermore, after eradication of *H. pylori*, antibody levels tend to fall to 50-60% of the pretreatment value within 6 months (15,16) ; therefore, unless the baseline and the posteradication sera can be compared directly, serological test cannot be used to assess the response to treatment.

Due to the greater sensitivity of the Polymerase Chain Reaction (PCR) compared with conventional isolation methods, it has been preferred in detection *H. pylori* DNA in faeces (17). PCR offers great promise as a highly sensitive and specific technique for the detection of *H. pylori* (18). However, the stool suspension must be pretreated to remove the PCR inhibitory substances and it is not known whether the specific DNA of *H. pylori* is derived from live or dead organisms. Although this method is not expensive, PCR is time-consuming and difficult to implement in routine clinical laboratories as it require specific equipment. However, due to its high sensitivity, PCR can detect very low levels of *H. pylori*, so contamination can give a false-positive result (19).

The development of a simple and rapid technique for the detection of *H. pylori* antigen in stool. A prospective study showed that HpSA was as sensitive as PCR in detecting in faeces and both were almost as sensitive as biopsy-based methods (20). Our results show that the HpSA is highly sensitive and specific for the detection of *H. pylori* infection. Similar results were reported in many papers (21-26).

One negative patient may be explained to our knowledge only by the limits of sensitivity and specificity of the test. Besides, all patients included in the study had not received recent antibiotic treatment.

The high positive correlation between the HpSA results and endoscopic procedures (rapid urease test and histopathological examination) for *H. pylori* infection, demonstrates the utility of this test as an alternative to invasive procedures. However, the accuracy of HpSA in detecting antigen after eradication needs further evaluation. The reports currently available in the literature, all published in abstract from, show conflicting results. In

most studies good sensitivities and specificities of the test, even after treatment, have been reported, but in others the discrepancy between HpSA, UBT and histopathology is high (27,28). Also the timing for monitoring *H. pylori* therapy with the HpSA test needs further evaluation (20,29-31).

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