Accurate Diagnosis of *Helicobacter pylori* infection by Stool Antigen Test and Six Other Currently Available Tests in Children

Yen-Hsuan Ni, MD, PhD, Jaw-Town Lin*, MD, PhD, Shiu-Feng Huang#, MD, PhD, Jyh-Chin Yang*, MD, and Mei-Hwei Chang, MD

Departments of Pediatrics, Internal Medicine*, and Pathology#

National Taiwan University Hospital,

College of Medicine, National Taiwan University,

Taipei, Taiwan

Corresponding author:

Mei-Hwei Chang, MD

Department of Pediatrics, National Taiwan University Hospital,

7, Chung-Shan South Road, Taipei, Taiwan 100

Tel: 886-2-23970800 ext 5131, 5509

Fax: 886-2-23938871

Email: mhchang@ha.mc.ntu.edu.tw

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ABSTRACT

Helicobacter pylori (*H. pylori*) infection has been associated with gastritis, peptic ulcer, and gastric malignancy. Invasive and noninvasive tests have been developed for the diagnosis of *H. pylori* infection. Since *H. pylori* infection is mostly acquired in childhood and adolescence, accurate diagnosis of the infection in the pediatric population is important. The noninvasive diagnostic methods are particularly feasible in children. We conducted a study to compare the invasive tests: culture, biopsy urease test (BUT), histology, and polymerase chain reaction (PCR) on gastric biopsy specimens, with noninvasive tests: serology, ^{13}C-urea breath test (^{13}C-UBT), and a new diagnostic modality: stool antigen test to diagnose *H. pylori* infection in children. A total of 53 symptomatic children were enrolled into this study and all had completed the seven diagnostic tests for *H. pylori*. Our results showed all the diagnostic tests except serology were excellent methods of diagnosing *H. pylori* infection in children. The diagnostic accuracy of the seven tests were as follows: stool antigen test 96.2%; BUT 96.2%; histology 98.1%; PCR 94.3%; culture 98.1%; ^{13}C-UBT 100%; and serology 84.9%. Stool antigen test, being highly sensitive and specific as shown in our data, will be potentially very helpful in diagnosing *H. pylori* infection in children.

List of Abbreviations

H. pylori	*Helicobacter pylori*
UBT	Urea breath test
PCR	Polymerase chain reaction
BUT	Biopsy urease test

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection has been linked to gastritis, [1-3] duodenal ulcer, [4,5] gastric cancer,[6] and mucosa-associated lymphoid tissue lymphoma. [7] It is now generally agreed that most *H. pylori* infections are acquired during childhood or adolescence, both in developing and developed countries. [8] *H. pylori* infection causes similar disease patterns in children as adults except for the rarity of malignancies. *H. pylori* infection was found in 90% of children with duodenal ulcers and in 25% of children with gastric ulcers.[5, 9] Acquisition of *H. pylori* at an early stage might increase the risk of developing gastric cancer.[10] The association of *H. pylori* infection to many clinical conditions in childhood increases the demand for an accurate diagnosis of *H. pylori* in childhood.

Two categories of diagnostic methods for *H. pylori* infection are defined: invasive tests to detect the microorganisms in biopsies sample of the gastric mucosa obtained through endoscopy, and noninvasive tests which obviate the need for endoscopy. These diagnostic tests have been applied to diagnose *H. pylori* infection in adults as well as in children. Deep sedation or even general anesthesia is sometimes required for endoscopy in children, while this procedure remains valuable in pediatric patients with symptoms suggesting peptic ulcer. Noninvasive tests, such as urea breath test (UBT), have been proved to be equally accurate in diagnosing and

following-up *H. pylori* infection in children.[11]

Although several studies have simultaneously compared the diagnostic accuracy among different methods in adult patients,[12-14] none have been conducted among pediatric patients. In this study, we have analyzed the diagnostic values of seven different tests for *H. pylori* infection, including culture, histology, biopsy urease test (BUT), polymerase chain reaction (PCR), ^{13}C-UBT, serology, and particularly, a newly developed stool antigen test.[15] This comparison may help to validate the diagnostic accuracy of the newly developed noninvasive stool antigen test. For those who are unable to cooperate to inhale and exhale on command, stool antigen test will be a good alternative for diagnosing *H. pylori* infection.

PATIENTS AND METHODS

Patients. From July 1, 1998 to August 30, 1999, fifty-three consecutive symptomatic children who received endoscopic examination in Department of Pediatrics, National Taiwan University Hospital, were enrolled into this study. There were 31 females and 22 males with a median age of 12 years, ranging from 10 to 14 years. All patients had recurrent abdominal pain and epigastralgia for at least one month. In addition, thirteen had hunger pain, five had family history of *H. pylori*-associated duodenal ulcer, and two were evaluated for coexistent anemia. A booklet with information concerning *H. pylori* infection and details of the diagnostic

procedures in this study was distributed to the patients and their parents. Informed consents were obtained from all of their parents. Subjects who could not tolerate endoscopy or who had been treated with antibiotics, H_2-blockers, or proton pump inhibitors in the recent six weeks were excluded from the study. If the subjects had ever been documented to have *H. pylori* infection, they would also be excluded from the study.

Endoscopy, biopsy, and biopsy urease test (BUT). All children received intramuscular injection of meperidine (2mg/kg, maximum 50 mg), and hyoscine butylbromide (0.5 mg/kg) before endoscopic examination. None of them was deeply sedated or underwent the endoscopic procedure under general anesthesia. Four biopsied specimens were taken at the antrum, 1-3 cm from the pyloric canal, and another three biopsied specimens were taken from the gastric corpus. These specimens were processed for BUT, culture, histologic examinations, and PCR. CLO test (Delta West, Bently, Australia) was adopted as BUT to detect the urease activity in biopsied specimens. The chromogenic reaction was read after one hour and 24 hours after of incubation.

Histologic examinations. The specimens were snapped to phosphate-buffered formalin, subjected to paraffin-embedding, and stained with hematoxylin and eosin. In addition, Diff-Quik stain was used to identify the presence of *H. pylori*.[16]

Polymerase chain reaction (PCR). The biopsied antrum tissues were minced and the DNA was extracted using the DNA extraction kit (Qiagen, Chatsworth, CA, USA). Each biopsied specimen was finally adjusted to a DNA concentration of 0.1 μg/μl. The nucleotide sequences of primers (first and nested) are from *H. pylori* urease C genome as previously described.[17] If there was no product after the first run of PCR, 1 μl of first run PCR product was subjected to the nested PCR. A DNA fragment containing the sequences of urease C gene is cloned, expressed, and serially diluted. This was used in the sensitivity assay as a positive control.

Bacterial culture. The procedures were described previously.[18] Briefly, biopsied specimens were immediately snapped into a brain-heart infusion broth. After grinding and transformation, they were streaked in the blood agar plate and incubated at 35-37 ℃ under microaerophilic conditions (5% O_2, 10%CO_2, 85% N_2) for 3-7 days. Organisms were identified as *H. pylori* on the basis of colony structure, results of Gram stain, and the production of urease, oxidase, and catalase.

^{13}C-Urea breath test (^{13}C-UBT). Infrared spectrophotometer (UBiT-IR200, Otsuka Electronics Co., Hirakata, Japan) was applied in this study.[19,20] It had been validated and correlated well with mass spectrometric analysis.[21] The patient fasted overnight before the test. The procedures were done following the manufacturer's instruction. Two bags of exhaled air were collected: one baseline and the other 15 minutes after

ingestion of powder containing 100mg ^{13}C-urea in 100 cc drinking water. The two bags were inserted into the inlets of infrared spectrophotometer and the increased percentage of $^{13}CO_2$ urea could be shown in five minutes. The cut-off point was 4.5‰.

Serology test. HEL-p II test kit (Amrad, Boronia, Victoria, Australia) for the determination of *H. pylori* IgG antibody was used in this study. This is an enzyme-linked immunoadsorbent assay which has been validated in a previous adult adenocarcinoma study.[22] Because there was no data available to document the optimal cut off point for *H. pylori* IgG antibody in children, we arbitrarily set it according to the manufacturer's instruction. All samples were tested in duplicate.

Stool antigen test. A fresh stool sample about the size of a peanut was collected and stored at –20°C for analysis as described previously.[23] *H. pylori* stool antigens were detected by a commercial kit (HpSA Microwell EIA, Meridian Diagnostic Inc, Cincinnati, OH, USA) using an enzyme-linked immunoadsorbent assay. This is an qualitative test utilizing a polyclonal rabbit anti-*H. pylori* antibody adsorbed to microwells. Diluted stool samples and a peroxidase-conjugated secondary polyclonal antibody were added to the microwells and incubated for one hour at room temperature. Reading of the results was based on spectrophotometric analysis. Reading of the results was based on spectrophotometric analysis: $OD_{450} < 0.14$ is negative, $OD_{450} > 0.16$ is positive, the value in between is equivocal. Equivocal results

should be repeated.

Standards for determination of H. pylori infection. All the tests were read independently without knowing the results of other tests. The final results of *H. pylori* infection status was defined as “infected” when the culture was positive or concordance of at least two of the three conventional tests (histology, BUT, ^{13}C-UBT). Patients with negative results on all the above four tests (culture, histology, BUT, and ^{13}C-UBT) were defined as “non-infected”. Those who could not be categorized into the above criteria were classified as “indeterminant” and excluded from the analysis.

RESULTS

Patients. A total of 53 children (31 females and 22 males) were enrolled into this study. Endoscopic diagnosis revealed active duodenal ulcer in three, duodenal ulcer scar with fold convergence in two, antral nodularity in 19, hyperemic mucosa at antrum and corpus in 19, and negative findings in the remaining 10 patients. The diagnostic results for culture, histology, BUT, and ^{13}C-UBT are described in Table 1. *H. pylori* status was diagnosed as “infected” in 27 cases, “non-infected” in the other 26 cases, and 0 in the “indeterminant” group.

Diagnostic accuracy of seven tests. The diagnostic accuracy of the seven tests were as follows: stool antigen test 96.2%; BUT 96.2%; histology 98.1%; PCR 94.3%; culture 98.1%; ^{13}C-UBT 100%; serology 84.9%. The sensitivity, specificity, predictive

values for a positive or negative test, and diagnostic accuracy were calculated and are shown in Table 2. For the stool antigen, there was no false positive and two cases were false negative. None of stool samples were read as “equivocal” in this study. Stool antigen test had a 92.6% sensitivity, 100% specificity, positive predictive value of 100%, and negative predictive value of 92.9%. BUT results showed two false negative cases and zero false positive. For the 25 cases BUT positive, 24 cases were read reactive within one hour (range 5-30 minutes) and only one case was read reactive 80 minutes after the biopsy. For the PCR, there was one false positive and two false negative result. For IgG anti-*H. pylori*, there were five false positive cases and three false negative cases.

DISCUSSION

To the best of our knowledge, this is the first report to simultaneously compare seven different diagnostic modalities for *H. pylori* in the pediatric population. In this study, we have demonstrated that current modalities to diagnose *H. pylori* infection in adults are similarly accurate in children, except for IgG antibody to *H. pylori*. Both invasive and noninvasive tests were satisfactory, with an accuracy rate about 95%.

Duodenal ulcer in children is highly associated with *H. pylori* infection.[5] The risk of developing gastric cancer is also relatively high if *H. pylori* infection is acquired at a young age.[10] In view of these critical issues, identification of childhood *H. pylori*

infection is important. To achieve this, accurate diagnosis of *H. pylori* infection in children is essential. At present, diagnosis of *H. pylori* infection in children still largely depends on the endoscopic biopsy of the gastric tissues for culture and urease test.[24] These methods were regarded as the gold standard. However, the invasive nature limits its wide use in children. Noninvasive diagnostic tests, including ^{13}C-UBT and serology, were recently developed and shown to be promising in establishing the diagnosis of *H. pylori* infection in children.

Stool antigen test is a new noninvasive test and was reported to have a comparable diagnostic value to any other diagnostic test in adults.[23,25] However, there have been no reports about the application of stool antigen test in children. Our data proves that this test works well in children. It gave 100% specificity and 92.6% sensitivity and the diagnostic accuracy is 96.2%. All the values were comparable to other tests (Table 2). Its advantage over ^{13}C-UBT for children is no need to exhale air, making it much easier for children who still cannot cooperate to inhale and exhale on command. Further investigation of stool antigen test in infants are needed to validate its use.

Non-dispersive infrared spectrometry was used to analyze $^{13}CO_2$ in this study. Although it requires more exhaled gas to be collected than the conventional $^{13}CO_2$ analysis by mass spectrometry, it is more convenient, less expensive, and highly

sensitive and specific in adult patients.[26] Rowland et al. reported that the sensitivity and specificity of ^{13}C-UBT could be as high as 100%, and 97.6% if the subjects were fasting.[27] Our data also supported that ^{13}C-UBT is of good diagnostic accuracy in school-aged children. It offers a feasible way for diagnosing *H. pylori* infection, and monitoring the therapeutic effects in children who can successfully follow the procedure of ^{13}C-UBT.

Most commercially available IgG antibody to *H. pylori* kits performed equally.[28] They have some pitfalls for diagnosis and therapeutic monitoring in children.[29,30] Probably due to the duration of infection and the difference in immunity and bacterial load, the antibody levels in children differ from the adults.[31] Moreover, spontaneous clearance of *H. pylori* may occur in some children with persistent antibody, thus resulting in false positive serological tests.[32,33] The serological tests are not good to detect previous infections since the titers decrease below the cut off value within months after eradication of *H. pylori* infection. Our data also demonstrated this test is of the least diagnostic value among the seven tests.

Noninvasive tests are not able to completely replace invasive tests such as endoscopic examinations. The majority of pediatricians will prefer to eradicate *H. pylori* infection according to the endoscopic and histologic examinations, rather than solely based on a single noninvasive test. The gastroduodenal pathology and the

severity of *H. pylori* infection still depends upon these invasive tests. Endoscopic examination is especially indispensable since the current guidelines for eradication therapy do not advocate treatment for all *H. pylori*-infected children.[34] At present, we suggest an endoscopic examination should be done before *H. pylori* eradication to define the gastroduodenal pathology both endoscopically and histologically.

In conclusion, this study demonstrated the currently available invasive (culture, BUT, PCR, histology) and noninvasive (UBT, and stool antigen test) diagnostic methods for *H. pylori* infection are excellent in children. Serology is the least valuable diagnostic method in children. Particularly, stool antigen test is potentially valuable in diagnosing and following up *H. pylori* infection in children because of its accessibility and noninvasiveness.

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Table 1. Concordance of diagnosis of H. pylori infection in 43 children by four diagnostic tests as gold standard*

Culture	Histology	BUT	^{13}C-UBT	*H. pylori* status	No. of patients
negative	negative	negative	negative	noninfected	26
positive	positive	positive	positive	infected	24
negative	positive	positive	positive	infected	1
positive	negative	negative	positive	infected	1
positive	positive	negative	positive	infected	1

gold standard: either culture is positive or concordance positive with at least two of the other three tests (Histology, BUT, and ^{13}C-UBT).

BUT: biopsy urease test

^{13}C-UBT: ^{13}C-urea breath test

Table 2. Sensitivity, specificity, and diagnostic accuracy of seven diagnostic tests for H. pylori infection in children

	BUT	Histology	PCR	Culture	^{13}C-UBT	IgG	SA
Sensitivity	92.6%	96.3%	92.6%	96.3%	100%	88.9%	92.6%
Specificity	100%	100%	96.2%	100%	100%	80.8%	100%
PPV	100%	100%	96.2%	100%	100%	82.8%	100%
NPV	92.9%	96.1%	92.6%	96.1%	100%	87.5%	92.9%
Accuracy	96.2%	98.1%	94.3%	98.1%	100%	84.9%	96.2%

PPV: positive predictive value, NPV: negative predictive value

BUT: biopsy urease test by CLO test

PCR: polymerase chain reaction

^{13}C-UBT: urea breath test by infrared spectrometer

IgG: serum IgG antibody to *H. pylori*

SA: stool antigen test