Stool antigen detection versus ^{13}C-urea breath test for non-invasive diagnosis of pediatric *Helicobacter pylori* infection in a limited resource setting

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Abstract

Introduction: The prevalence of childhood infection with *Helicobacter pylori* is high, especially in developing countries. Non-invasive methods for detection of infection in children should be inexpensive, easy to perform, well tolerated and have a high diagnostic accuracy. We aimed to compare the reliability, specificity and sensitivity of the *H. pylori* stool antigen (HpSA) test with the ^{13}C-urea breath test (^{13}C-UBT) for the diagnosis of *H. pylori* infection in a limited resource setting.

Material and methods: The stool samples of 60 symptomatic and dyspeptic children with a mean age of 7.2 ±3.7 years (2–15 years) were evaluated using the rapid One step HpSA test by lateral flow immunoassay. The ^{13}C-UBT was used as the gold standard method for the diagnosis of *H. pylori* infection.

Results: The HpSA test detected *H. pylori* antigen in 34 out of 38 positive patients with 4 false-negatives (sensitivity 89.5%, 95% confidence interval (CI): 75.2–97.1%), while 21 patients had true-negative results and one false-positive (specificity 95.5%, 95% CI: 77.2–99.9%), with a strong measure of agreement between the HpSA test and the ^{13}C-UBT (κ = 0.83, 95% CI: 68–97%, p < 0.001). It had a positive predictive value of 97.1% (95% CI: 85.1–99.9%), a negative predictive value of 84% (95% CI: 63.9–95.5%) and an accuracy of 91.7%.

Conclusions: The rapid lateral flow HpSA test is a reliable method for the primary diagnosis of *H. pylori* infections in children, though not as accurate as the ^{13}C-UBT. It is more affordable, simpler to perform and more tolerable, representing a viable alternative, especially in developing countries.

Key words: *Helicobacter pylori*, *H. pylori* stool antigen test, ^{13}C-urea breath test.

Introduction

Helicobacter pylori infection is one of the most common bacterial infections in humans, having a worldwide distribution and affecting nearly 50% of the world's population [1]. The majority of persons infected with *H. pylori* develop chronic gastritis, but they are often asymptomatic [2]. The infection is mainly acquired during childhood or adolescence [3]. This acquisition early in life has been reported to increase the risk of peptic ul-

cer [4] and gastric cancer in adulthood [4, 5]. *Helicobacter pylori* is the main cause of both duodenal and peptic ulcers and is associated with the risk of developing gastric cancer.

The prevalence of *H. pylori* is inversely related to socioeconomic status [6], and in many developing countries it is over 80%, which is substantially higher than in industrialized countries, where it is under 40% [7]. The prevalence of infection with *H. pylori* among Egyptian children aged 2–17 years in the city of Cairo is 46% [8].

In view of all the aforementioned issues, identification and accurate diagnosis of *H. pylori* infection in children are essential. Several invasive and non-invasive diagnostic tests are used for the detection of *H. pylori* infection [9]. Endoscopic diagnosis coupled with biopsy of the gastric tissue for culture and the urease test is considered rather inconvenient, invasive and expensive, with potential complications [10]. The ^{13}C-urea breath test (^{13}C-UBT) has largely replaced endoscopy and become the gold standard diagnostic investigation for *H. pylori* infection [11]. The ^{13}C-UBT is non-invasive, accurate and relatively expensive. It requires mass spectrometric analysis, which may not be available at small centers in developing countries with limited resources. However, the ^{13}C-UBT can now be performed using a more compact infrared spectrophotometer, which is less expensive and easier to use than mass spectrometry [12]. Recently, a potential role for alcohol dehydrogenase class IV isoenzyme as a marker for *H. pylori* infection has been suggested, with a diagnostic sensitivity and specificity of 88% and 90%, respectively [13].

Non-invasive methods for detection of *H. pylori* infection are required to study its incidence, transmission, and clearance. They should be easy to perform and inexpensive, have a high diagnostic accuracy and be well tolerated, especially in infants and toddlers. Detection of *H. pylori* stool antigen (HpSA) is becoming an alternative, non-invasive, simple and cost-effective diagnostic test; however, its accuracy in developing countries, particularly in children, has not yet been well-established. To the best of our knowledge, comparison between the ^{13}C-UBT and HpSA has not been reported in Egyptian children. The aim of this study was to compare, prospectively, the reliability, specificity and sensitivity of HpSA testing, using rapid lateral flow immunoassay, with the ^{13}C-UBT as the gold standard method for the diagnosis of *H. pylori* infection in a cohort of symptomatic Egyptian children, in a limited resource setting.

Material and methods

Patient population

We prospectively enrolled 60 symptomatic and dyspeptic children selected from the tropical pediatric clinic at Cairo University Children's Hospital, Cairo, Egypt, from June 2012 to March 2013. They were 30 males and 30 females. Written consent was obtained from the parents of eligible children. The study was ethically approved by the Institutional Review Board of the Faculty of Medicine, Cairo University. Patients enrolled were complaining of recurrent abdominal pain, persistent vomiting, hematemesis and growth failure, either separately or in different combinations. Children receiving antimicrobial therapy or proton pump inhibitors within the preceding 1 month were excluded from the study. All patients underwent careful history taking, thorough clinical examination and laboratory investigations including complete blood picture, urine and stool analysis, ^{13}C-UBT and HpSA.

^{13}C-UBT

The ^{13}C-UBT required that the child be brought to the laboratory following an overnight fast to collect a baseline sample of expired breath in a double way test bag. The child was then fed 50 mg of ^{13}C-labeled urea substrate on a citric acid base (fresh pure orange juice) as the ^{13}C-UBT meal. Thirty minutes after ingestion, a breath sample was collected again and analyzed using isotope ratio mass spectrometry (12/13 CO_2-breath test analyzer FANci2, Fischer Analysen Instrumente GmbH, Steingrund Dreieich, Germany). The test was considered positive when the delta over baseline (DOB) value was > 4.0%.

HpSA test

The HpSA test was less time-consuming. Stool specimens were tested using the One step *H. pylori* antigen test device (IHP-602, ACON Laboratories, Inc., San Diego, USA; Prime diagnostics, São Paulo, Brazil) according to the manufacturer's instructions (94.9–100% sensitivity and 95.1–100% specificity, according to the manufacturer). The one step test is a qualitative, lateral flow immunoassay for the detection of HpSA in human fecal specimens. In this test, the membrane is pre-coated with anti-*H. pylori* antibodies in the test line region. During testing, the specimen reacts with the anti-*H. pylori* antibodies. The mixture migrates upwards on the membrane by capillary action and reacts with the anti-*H. pylori* antibodies on the membrane. This generates a colored line across the central window of the cassette. The presence of this colored line in the test region T indicates a positive result, while its absence indicates a negative one. To serve as a procedural control, a colored line should always appear in the control region C, indicating that the proper volume of the specimen has been added.

In order to obtain maximum antigens, we collected a sufficient quantity of feces (1–2 ml or 1–2 g) in a clean, dry specimen container. The assay was performed within 6 h after stool collection. Small samples collected from three different sites of the stool specimen were transferred into a specimen collection tube containing extraction buffer and then shaken vigorously. The tube was left for 2 min, then two full drops of the extracted specimen were transferred into the specimen wells of the test device. Results were read after 10 min of incubation at room temperature. Based on the appearance of colored lines across the central window of the cassette, the appearance of two lines, C and T, indicated a positive result. A pale colored line in T was also considered as positive. The appearance of only one line in C indicated a negative test.

Statistical analysis

Data analysis was carried out using the Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL, USA) version 16.0 for Windows. Mean ± standard deviation (SD) was calculated for quantitative data and percentage for categorical variables. Numbers and percentages were compared by the χ^2 test. The sensitivity, specificity, and positive and negative predictive values were calculated, and the κ coefficient was used as a measure of agreement for categorical data.

Results

The ages of the 60 children enrolled in this study ranged from 2 to 15 years, with a mean age ± SD of 7.2 ±3.7 years. The performance of the HpSA test was assessed using the ^{13}C-UBT as the gold standard method, to define *H. pylori*-positive and -negative groups. Out of the 60 pediatric patients studied, 38 had a positive ^{13}C-UBT and were therefore considered as having *H. pylori* infection. The HpSA test was positive in 34 (89.5%) of the 38 patients (true-positive) and negative in 4 (false-negative) (Table I), indicating a sensitivity of 89.5% (95% confidence interval (CI): 75.2–97.1%) (Table II). The remaining 22 children who were negative according to the ^{13}C-UBT were considered *H. pylori*-negative and were diagnosed as having functional dyspepsia. The HpSA test was negative in 21 (95.5%) cases (true-negative) and positive in 1 (false-positive) (Table I), denoting a specificity of 95.5% (95% CI: 77.2–99.9%) (Table II). Considering that there were 34 true-positive and 1 false-positive test results, the positive predictive value (PPV) was 97.1% (95% CI: 85.1–99.9%); and as there were 21 true-negative and 4 false-negative tests, the negative predictive value (NPV) was 84% (95% CI: 63.9–95.5%) (Table II). Our findings revealed a strong measure of agreement between the HpSA test and the ^{13}C-UBT (κ = 0.83, 95% CI: 68–97%, $p < 0.001$).

Table I. Results of HpSA test in comparison with UBT

Fecal test	UBT	
	Positive (*N* = 38) *n* (%)	Negative (*N* = 22) *n* (%)
HpSA:		
Positive	34 (89.5)	1 (4.5)
Negative	4 (10.5)	21 (95.5)

HpSA – Helicobacter pylori stool antigen, UBT – urea breath test.

The HpSA test gave 34 true outcomes per 60 tested, for a cost of US $431.65 for 60 tests, and a mean cost of $12.70 per true positive test, while the ^{13}C-UBT had 38 true outcomes, for a cost of $906.47 for 60 tests and a mean cost of $23.85 per true positive test (Table III). The incremental cost-effectiveness ratio (ICER) for the ^{13}C-UBT when compared with the HpSA test was $118.71; this is the difference in cost ($906.47 minus $431.65) divided by the gain of 4 true positive outcomes.

Discussion

The economic, ethical, and public health implications of the presence of *H. pylori* infection in

Table II. Performance of HpSA test as compared with UBT

Fecal test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
HpSA	89.5	95.5	97.1	84.0	91.7

HpSA – Helicobacter pylori stool antigen, NPV – negative predictive value, PPV – positive predictive value, UBT – urea breath test.

Table III. Cost-effectiveness of HpSA test and UBT

Test	Cost/60 tests (US$)	Effectiveness (number of true outcomes)	Mean cost/true positive test (US$)	ICER (US$)
HpSA	431.65	34	12.70	
UBT	906.47	38	23.85	118.71

HpSA – Helicobacter pylori stool antigen, ICER – incremental cost-effectiveness ratio, UBT – urea breath test.

children have motivated the search for accurate, non-invasive tests [14]. The ease of administration and ability to test large numbers of children in a short time have made non-invasive tests the assays of choice for diagnosing *H. pylori*, especially in epidemiologic studies. In our study, out of the 60 dyspeptic children, 38 (63.3%) were diagnosed as *H. pylori*-positive by the ^{13}C-UBT.

According to Baumann [15], HpSA testing is approved by the US Food and Drug Administration (FDA) for use as a non-invasive diagnostic test of *H. pylori* infection and as a test to monitor the response to treatment. We observed a high sensitivity, specificity and overall accuracy for the HpSA test (89.5, 95.5 and 91.7%, respectively), showing a strong measure of agreement between the HpSA test and the ^{13}C-UBT (κ = 0.83, 95% CI: 68–97%, $p < 0.001$). Our findings are consistent with those of Silva *et al*. [2], who tested the rapid lateral flow chromatography stool antigen assay using the ^{13}C-UBT as the gold standard method and detected a sensitivity and specificity of 88% and 87.5%, respectively, presenting a substantial agreement with the breath test (κ = 0.75). The stool antigen test may be performed by conventional enzyme immunoassay (EIA) using polyclonal antibodies, showing a sensitivity and specificity of 88.9% to 98.3% and 94% to 100%, respectively, in children [10, 16–18], and a sensitivity and specificity of 79% to 91.9% and 80.5% to 100%, respectively, in adults [19–21]. A slightly lower sensitivity and specificity of 66.7% and 78.9%, respectively, were detected by Syam *et al*. [22] in adults.

The false-negative test results of HpSA might have been due to low colonization of bacteria in the stomach leading to a low concentration of antigens of *H. pylori* in the feces and the inability to react in the test [9]. The false-positive result might have been caused by the presence of the coccoid form of *H. pylori*, i.e., the morphologic manifestation of bacterial cell death, which does not denote an infection [23]. It is important to note that the possibility of external contamination could not be excluded, although stools were collected in clean sterile containers. It is also hard to rule out the positive antigen detection from the intestinal flora due to the stool sample source. The cross-reaction among the polyclonal antibodies of the test with antigens of bacteria from the intestinal flora may explain the false-positive result.

The diagnostic accuracy of the HpSA test, particularly the sensitivity, is reduced by upper gastrointestinal bleeding, and a negative test result should be confirmed by further diagnostic methods [24]. When the rapid lateral flow fecal antigen tests were evaluated in adults, the incubation time was observed to be an important factor for the reading of the result. Readings at 30 min (76.9%) and 60 min (78.6%) had higher sensitivity than after 20 min (59.1%), suggesting a new reading strategy to increase the sensitivity: the first interpretation at 15–20 min, then a longer incubation time of 30 min when negative results occur after 20 min, with a possible interpretation at a final reading of 60 min for the very low percentage of undetermined results at 30 min [25].

The stool antigen test by lateral flow immunoassay performed well in children. It is readily available in many hospitals and medical centers, can be performed in any laboratory and is useful for small laboratories that work with few samples or do not have the equipment for performing EIA. It is faster than conventional EIA, which takes more than two hours to be performed [26]. On the other hand, the ^{13}C-UBT, while being more accurate than the fecal test, was time-consuming and more cumbersome to perform, as collection of air was very difficult in very young children. Thus, it was not well tolerated by pediatric patients. The lack of availability of the ^{13}C-UBT due to the high cost of the test and, in particular, the need for expensive analytical instrumentation limits the usefulness of this method, making the rapid lateral flow stool antigen test cheaper and more affordable. Single testing, as recommended by the manufacturer, is a safe approach and will reduce the costs of the test. Thus, the stool antigen test seems to be a cost-effective method. This is in agreement with other investigators [27] who, on the basis of cost-effectiveness analysis, stated that the fecal antigen test, when compared to serology and the UBT, is the preferable strategy for diagnosis of *H. pylori* in primary care.

In conclusion, the lateral flow HpSA test is a reliable method for the primary diagnosis of *H. pylori* infections in children, though not as accurate as the ^{13}C-UBT. It is more affordable, more practical and simpler to perform, and more tolerable. It represents a viable alternative to the ^{13}C-UBT, especially in developing countries.

Conflict of interest

The authors declare no conflict of interest.

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