Original Article

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Stool Antigen Tests for the Detection of *Helicobacter Pylori* in Children

Abolfazl Iranikhah[1], MD; Mohammad-Reza Ghadir[2], MD; Saeed Sarkeshikian[2], MD; Hosein Saneian[3], MD; Akram Heidari[4], MD; Mohadeseh Mahvari[5], MD

1. Department of Pediatric Gastroenterology, Qom University of Medical Sciences, Qom, Iran
2. Department of Gastroenterology, Qom University of Medical Sciences, Qom, Iran
3. Department of Pediatrics, Isfahan University of Medical Sciences, Isfahan, Iran
4. Department of Social Medicine, Qom University of Medical Sciences, Qom, Iran
5. Qom University of Medical Sciences, Qom, Iran

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Abstract

Objective: Reliable non-invasive methods for detection of *Helicobacter pylori* (*H. pylori*) infection are required to investigate the incidence, transmission, and clearance of infection in childhood. Detecting bacterial antigens in stool offer an alternative noninvasive diagnostic test. However its accuracy in developing countries is not well established. The aim of this study was to evaluate the performance of stool antigen test for H *pylori* in Iranian children with recurrent abdominal pain necessitating endoscopy.

Methods: One hundred three children enrolled in this study. Endoscopy and biopsy was done on all patients providing a criterion standard for validation of the *H. pylori* stool antigen (HpSA) tests. The presence of *H. pylori* organisms in stool was determined by an enzyme-linked immunosorbent assay using a commercially available polyclonal antibody. HpSA sensitivity, specificity, and positive and negative likelihood ratios were determined with reference to the results of cultures of gastric biopsy.

Findings: Of the 103 children tested 41 (39.8%) and 39 (37.8%) were positive for *H. pylori* according to the results of cultures of gastric biopsy and HpSA, respectively. The sensitivity, specificity, and positive and negative likelihood ratios of HpSA were found to be 85%, 93%, 89.7%, and 90%, respectively.

Conclusion: In this pilot study, a low-cost and rapid diagnostic technique, stool antigen test proved to be highly sensitive and specific for detecting H *pylori* infection in children with recurrent abdominal pain. Our results are comparable to those reported elsewhere in children and demonstrate that the HpSA test can replace endoscopy and biopsy for detecting *H. pylori* infection.

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Key Words: Helicobacter Pylori; Stool Antigen Tests; Children

Introduction

Helicobacter pylori (*H. pylori*) infection is acquired mainly in childhood, especially in developing countries[1], where the influence of socioeconomic factors on the prevalence of *H. pylori* infection has been shown[1-4]. There is a great contrast between developed countries, where only few children are infected and developing countries, where most children reach adulthood being *H. pylori* positive. In underdeveloped countries, up to 66% of individuals harbor the organism[5,6]. In developing regions, for socioeconomic reasons, most infected children are not diagnosed and/or treated for *H. pylori* infection.

Although one important controversy relates to the presence of recurrent abdominal pain in children, where an important association was

*** Corresponding Author;**
Address: Hazrate Masoume Hospital, Qom, Iran
E-mail: iranikhah@sina.tums.ac.ir

observed between recurrent abdominal pain and *H. pylori* infection in some populations[7-11], some studies show *H. pylori* infection is probably not a cause of recurrent abdominal pain in children[12].

Many investigators have studied the criteria for diagnosis and treatment of children infected by *H. pylori*, but association of symptoms with *H. pylori* infection in children presenting with nonulcer-dyspepsia is controversial[13-15].

The criterion standards for diagnosis of active *H. pylori* infection are methods based on endoscopy and biopsy[16]. Diagnosis of *H. pylori* infection can be made with both invasive and noninvasive tests. Invasive tests include histology, culture and rapid urease test which require endoscopy to obtain biopsies of the gastric mucosa which is expensive and inconvenient. Noninvasive tests, which are based on analysis of samples of breath, blood, or stool, have been developed. Urease breath test (UBT) has been shown to be excellent in performance, but is expensive, may involve a visit to the hospital, and may be complicated to perform[17]. Among the noninvasive methods, serological tests cannot be applied to young children because of low sensitivity.

Reliable non-invasive methods for detection of *H. pylori* infection are required to investigate the incidence, transmission, and clearance of infection in childhood. Detecting bacterial antigens in stool offer an alternative noninvasive diagnostic test. Its performance in children and teenagers has been tested in some developed countries, showing a sensitivity and specificity above 90%[18]; however, its accuracy in developing countries is not well established. The aim of this study was to evaluate the performance of stool antigen test for *H pylori* in Iranian children with recurrent abdominal pain.

Subjects and Methods

One hundred three children (aged 4-15 y, 47 females, 56 males) who underwent upper gastrointestinal endoscopy due to recurrent abdominal pain were enrolled in this study. Exclusion criteria included receiving antibiotics, H2 antagonists, or proton pump inhibitors within the preceding 3 months. Recurrent abdominal pain is defined as paroxysmal abdominal pain in children between the ages of 4 and 16 years that persists for more than 3 months and affects normal activity.

The study was approved by the Ethics Committee of the Qom University of Medical Sciences.

Endoscopy and biopsy was done on all patients providing a criterion standard for validation of the *H. pylori* stool antigen (HpSA) tests. Gastric biopsy specimens were collected from the same location in the antrum of the stomach. Specimens were used for the culture of *H. pylori*. *H. pylori* was cultured on Columbia agar supplemented with 7% horse blood at 37°C for 4 to 6 days under microaerophilic conditions. Isolated strains were identified as *H. pylori* by Gram stain, morphology, and positive urease, oxidase, and catalase tests[19].

Stool samples were collected from each participant before endoscopy. The samples were stored at –20°C until analyzed. The presence of *H pylori* organisms in stool was determined by an enzyme-linked immunosorbent assay using a commercially available polyclonal antibody kit (Astra SRL, Via Ciro Menotti, Milano, Italy). Stool samples were diluted, added to antibody-coated microwells, and incubated. *H pylori*-specific polyclonal antibodies conjugated to horseradish peroxidase were added, incubated, and washed before peroxidase was added; a visible blue reaction indicated the presence of *H pylori*.

HpSA sensitivity, specificity, and positive and negative likelihood ratios were determined with reference to the results of cultures of gastric biopsy specimens.

Data were analyzed by descriptive analysis using SPSS 18. Chi-square test was used to compare categorical data.

Findings

One hundred three patients were analyzed. There were 56 (54/4%) male and 47 (45/6%) female patients, with a mean age of 8.32 years. *H pylori* infection was detected in 41/103 (39.8%) of the patients.

39 (37.8%) of the 103 children tested, including 22 (56.4%) females and 17 (43.6%) males with

mean age of 9.14 years, were positive for *H pylori* according to the results of HpSA test. No statistically significant difference was found between sex and *H. pylori* infection (*P*=0.4).

Of 41 patients who were positive by endoscopy-based tests 35 patients were also positive by the HpSA test; of 62 patients diagnosed as negative by endoscopy-based tests, 58 patients were also negative by the HpSA test.

In this context, qualitative enzyme-linked immunosorbent assay polyclonal antibodies were done for determination of the bacterial antigen in human stool. According to the detailed information provided within the commercial kit, the sensitivity, specificity, and positive and negative likelihood ratios of the test used in our study were 85%, 93%, 89.7%, and 90%, respectively. Table 1 shows the cross-classification of the defined standard for *H. pylori* infection status of these 103 children with the results of the cultures of gastric biopsy specimens.

Table 1: *H pylori* status by cultures of gastric biopsy specimens and HpSA

HpSA status	Cultures of gastric biopsy specimens		
	Positive	Negative	Total
Positive	35	4	39
Negative	6	58	64
Total	41	62	103

HpSA: H *pylori* stool antigen

Discussion

Methods for the detection of *H. pylori* infection are classically divided into invasive and noninvasive. No single test is a fully reliable method for detection of *H. pylori* in all instances. Even histology is not positive in 100% of infected children[20]. The current criterion standard for diagnosing *H. pylori* infection is endoscopic biopsy of the gastric tissue for rapid urease test, histology, and culture. However, such an invasive procedure has major disadvantages of anesthesia, discomfort, and the possibility to be a source of ethical problems.

Based on the recommendations of the European task force for *H. pylori* infection in children[16] the authors stated that upper gastrointestinal endoscopy with gastric biopsy is the method of choice for symptomatic children, and noninvasive tests to screen children with abdominal pain are not recommended. However, in developing countries the infection rate in infants and young children is high[6,21] and in such situations, an invasive test in children may not be suitable.

Although studies have suggested that the UBT is the most reliable noninvasive test in the general population, it may be less efficient in pediatric patients[17,22]. And despite the excellent diagnostic accuracy of the UBT, it requires special instrumentation and specialized staff.

Several noninvasive diagnostic methods based on the detection of fecal antigens of *H. pylori* have been developed. At present, their diagnostic accuracy is controversial[23,24]. Three different forms of the test have been commercialized, an enzyme linked immunosorbent assay using polyclonal antibodies, an enzyme-linked immunosorbent assay using a monoclonal antibody, and a rapid immunochromatographic test using a monoclonal antibody.

The United States and European authorities approved the stool antigen tests as a reliable tool in the primary diagnosis and also in the monitoring of post treatment outcome of *H. pylori* infection[24,25].

In the present study, HpSA test had sensitivity and specificity of 85% and 93%, respectively for *H. pylori* screening in children with abdominal pain. Most previous studies in adults and children have compared the efficacy and accuracy of the polyclonal HpSA with the various invasive and non-invasive tests used for diagnosing *H*. pylori infection[26-32]. We demonstrated high sensitivity, specificity and likelihood ratios for the polyclonal stool antigen compared with invasive test in the diagnosis of *H. pylori* infection in children. The sensitivity and specificity of the stool test are reported to be over 90% in children with gastrointestinal symptoms[33-35].

Although many investigators have reported high sensitivities and specificities of HpSA tests in children, the opinions on the efficacy of the HpSA test in patients with abdominal pain in developing countries are conflicting. The accuracy of the stool antigen test has been confirmed in children, and this may be the optimal test to screen and confirm success of eradication therapy[27,30]. The test may

also be recommended for children when endoscopic examination is infeasible[27,30] and for post treatment eradication testing in children[19]. The stool antigen test was found to be a useful method to screen children with abdominal pain for *H. pylori* infection in developing countries.

Stool antigen test, which detects present but not previous infection of *H. pylori*, would be applicable in mass survey. Usefulness of stool antigen tests for the screening of gastric cancer should be examined. However there is a need for further studies with a greater number of patients for evaluation of its accuracy in children of developing countries.

Conclusion

In this pilot study, a low-cost and rapid diagnostic technique, stool antigen test, proved to be highly sensitive and specific for detecting *H. pylori* infection in children with recurrent abdominal pain. Our results are comparable to those reported elsewhere in children and demonstrate that the HpSA test can replace endoscopy and biopsy for detecting *H. pylori* infection.

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Conflict of Interest: None

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