Diagnostic value of stool antigen and antibody tests for *Helicobacter pylori* infection in Turkish children with upper gastrointestinal complaints before and after eradication

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The aim of this study was to evaluate the diagnostic value of *Helicobacter pylori* stool antigen (HpSA) and serologic tests before and after eradication therapy for *H. pylori* in Turkish children in our region with upper gastrointestinal complaints. In this study, 87 children with upper gastrointestinal complaints and 95 children with various symptoms without gastrointestinal complaints were enrolled. *H. pylori* infection was detected by urea breath test (UBT). HpSA and *H. pylori* immunoglobulin G (HpIgG) tests were applied to all the children. Eradication treatment was given to the 34 *H. pylori*-positive children. The UBT was positive in 43 of 87 children (49.4%) with upper gastrointestinal complaints. The sensitivity and specificity of the HpSA in children with upper gastrointestinal complaints were 86% and 84.1%, respectively, and those for the HpIgG were 76.7% and 90.9%, respectively. After eradication, the overall HpSA test sensitivity and specificity values were detected as 88.9% and 84%, respectively, and those for the HpIgG were 77.8% and 36%, respectively. The HpSA test is highly sensitive and specific for the diagnosis of *H. pylori* and for confirming eradication in Turkish children with upper gastrointestinal complaints. However, serology is not a reliable method for the diagnosis of *H. pylori* infection or for confirming eradication in children.

Key words: *Helicobacter pylori,* stool antigen test, serology, child.

Helicobacter pylori (*H. pylori*) is one of the most common bacterial pathogens in humans. *H. pylori* infection is now recognized as a worldwide problem. It is well known that childhood is an important period for acquisition of *H. pylori* infection. Although, in most children, the presence of *H. pylori* infection does not lead to clinically apparent disease, *H. pylori* is considered to be the major cause of chronic gastritis and duodenal ulcer in childhood. However, the association between *H. pylori* and non-ulcer dyspepsia, recurrent abdominal pain, gastric outlet obstruction, and extraintestinal manifestations is still controversial[1]. The risk factors described for infection include residence in a developing country, poor socioeconomic conditions, family overcrowding, and ethnic or genetic predisposition[2].

H. pylori infection can be diagnosed by several invasive and noninvasive techniques. The invasive diagnostic procedures require endoscopic manipulation and tissue extirpation. Invasive tests include biopsies and histology, rapid urease testing, bacterial culture and polymerase chain reaction of bacterial DNA. The gold standard for the diagnosis of *H. pylori* infection is generally endoscopic biopsy-based tests[2,3]. However, these tests have their own disadvantages such as being invasive, expensive or inapplicable in childhood[4]. Thus, the use of noninvasive tests to diagnose *H. pylori* infection is becoming more frequent. The noninvasive tests include the urea breath test (UBT), serum and whole blood antibody, saliva antibody, urine antibody, and *H. pylori* stool antigen (HpSA)[2]. However, choosing the right noninvasive test

is usually not easy. Several factors, such as sensitivity, specificity, positive and negative predictive values, practicability, availability, and cost-effectiveness of the methods, play very important roles in this choice.

The UBT is noninvasive and has high sensitivity and specificity (>95%) both in adults and children (2,5). Although the UBT has a good sensitivity for the diagnosis of the infection in all ages, a low specificity in very young children compared to endoscopic biopsy-based tests has been found[6]. On the other hand, among these noninvasive tests, HpSA had the highest sensitivity and specificity (94% and 81%, respectively)[1]. The HpSA test is cheap and easy to perform and does not require expensive equipment. The basic technique in a conventional HpSA test is the enzyme-linked immunosorbent assay (ELISA) method (HpSA ELISA), and HpSA ELISA has been applied in both adults and children with high diagnostic accuracy[7]. The polyclonal HpSA test has revealed good overall performance in diagnosing *H. pylori* infection and evaluating the success of eradication therapy. Nevertheless, lower accuracy compared to the results seen with UBT after eradication therapy is a limiting factor for this test. A recently developed monoclonal HpSA test exhibited significantly higher sensitivity than the polyclonal test (94.3% versus 80.0%)[8]. Serologic tests have low diagnostic accuracy, particularly in children[9]. Antibody cut-off values are not determined in the pediatric population. Antibody positivity progresses with age in childhood. Furthermore, antibody levels persist in the blood for a long time after the treatment, so persistent antibodies will lead to false-positive results[10].

The aim of the present study was to evaluate the diagnostic value of HpSA and serologic tests before and after eradication therapy for *H. pylori* in children in our region with upper gastrointestinal complaints using the UBT test for comparison.

Material and Methods

Subjects

In this study, between January 2003 and December 2005, 87 children who visited the Department of Pediatric Gastroenterology with upper gastrointestinal complaints and 95 children who visited the Department of Pediatrics with various symptoms without gastrointestinal complaints were enrolled. The age range of the children was 6–18 years (mean ±SD: 10.9±3.6 years).

Children with upper gastrointestinal complaints who had chronic reccurrent abdominal pain (at least 3 pain episodes in the previous 3-month period) and/or who had dyspeptic symptoms such as upper or epigastric abdominal pain or discomfort, bloating, nausea, vomiting, early satiety, postprandial abdominal distention, and nausea or vomiting for at least the previous three months were included in the study.

The children with upper gastrointestinal complaints who had additional symptoms, such as diarrhea, urgency or dysuria, were excluded from this study. Children who had received antibiotics, histamine-2 blockers, non-steroidal anti-inflammatory drugs, or proton pump inhibitors within four weeks before the performance of *H. pylori* tests and who had chronic diseases such as chronic renal failure, diabetes mellitus or celiac disease were also excluded. In addition, children who had pathological test results for differential diagnosis of abdominal pain, such as complete blood count, urinalysis, blood glucose, serum electrolytes, liver function tests, kidney function tests, parasite examination in stool, and abdominal ultrasonography were excluded.

H. pylori infection was detected by UBT (*Helicobacter Test Infai, Bochum, Germany*) in children with upper gastrointestinal complaints. HpSA (*Diagnostic Bioprobes Srl, Milan, Italy*) and *H. pylori* immunoglobulin G (HpIgG) (*Radim, Rome, Italy*) tests were applied to all the children. A two-week treatment regimen of amoxicillin, clarithromycin and lansoprazole was given to the 34 *H. pylori*-positive children with upper gastrointestinal complaints. These children were re-evaluated 4-6 weeks after treatment with UBT, HpSA and HpIgG tests.

Urea Breath Test

In the children with upper gastrointestinal complaints, the UBT was started with the collection of the baseline breath sample after at least 12 hours fasting. After drinking 200 ml pure orange juice, the test solution (13C-labeled urea; 45 mg for children, 75 mg for

adolescents) was prepared. Thirty minutes after administration of the test solution, another breath sample was collected. The analysis of the breath samples was carried out by means of Isotope Ratio Mass Spectrometry. The ^{13}C-UBT was considered positive when delta over baseline was greater than 4.0%. For ethical reasons, UBT was only performed in children with upper gastrointestinal complaints.

H. pylori Stool Antigen Test

The HpSA test was performed by ELISA method. This test utilizes a plurality of monoclonal anti-*H. pylori* capture antibodies adsorbed to microplate wells. The samples were stored at

-70°C until the assay. After emulsifying a portion of stool into 200 µl sample diluents in a test tube, 50 µl of the diluted stools and controls were transferred into microwells. Enzyme conjugate (1 ml) was added to each well and the well was sealed. The mixture was then incubated at room temperature for 1 hour (h). After washing five times with the wash buffer, two drops of the substrate solution were added to each well and incubated at room temperature for 10 minutes (min). One drop of stop solution (sulphuric acid) was added to each well, and the results were spectrophotometrically read at dual wavelengths of 450 nm. According to the manufacturer's instructions, an absorbance at 450 nm of <0.100 was defined as negative and of >0.100 was defined as positive, respectively.

H. pylori IgG Test

Venous blood of patients was collected; serum was separated and stored at -20°C for further processing. HpIgG was measured in all subjects using ELISA method. Manufacturer's instructions were followed and a titer under the cut-off value was defined as negative while a titer above the cut-off value was defined as positive.

Statistical Methods

Statistical analysis was performed by SPSS 13.0 computer program. The children with upper gastrointestinal complaints and without upper gastrointestinal complaints were compared using the χ^2 test. Furthermore, the comparison to children with upper gastrointestinal complaints before and after eradication therapy was accomplished by the χ^2 test. P values less than 0.05 were regarded as statistically significant.

Ethical Clearance

The design of this study was approved by the ethics committee of the Medical Faculty of Celal Bayar University, Manisa. Written informed consent was obtained from the children and/or the parents prior to entry into this study.

Results

The UBT was performed in all children with upper gastrointestinal complaints for the diagnosis of *H. pylori* infection. The UBT was positive in 43 of 87 children (49.4%) with upper gastrointestinal complaints (18 boys, 25 girls; mean±SD: 11.9±2.8 years) and was negative in 44 of 87 children (50.5%) with upper gastrointestinal complaints (12 boys, 32 girls; mean±SD: 9.4±3.4 years). The mean age of the children without upper gastrointestinal complaints was 10.1±3.4 years.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false positivity (FP), and false negativity (FN) values of HpSA and HpIgG test in children with upper gastrointestinal complaints are shown in Table I.

In *H. pylori*-positive children with upper gastrointestinal complaints, sensitivity, specificity, PPV, NPV, FP, and FN values of HpSA and HpIgG test in determination of eradication after treatment are shown in Table II.

With the HpSA test, *H. pylori* positivity in children with upper gastrointestinal complaints was found to be 50.5% (n=44) and *H. pylori* positivity in children without upper gastrointestinal complaints was found to be 18.9% (n=18). After the treatment, eradication was achieved in 73.5% and compliance to treatment was 85.3% in *H. pylori*-positive children with upper gastrointestinal complaints. Non-compliance to treatment was observed in 55.5% of children in whom *H. pylori* eradication could not be achieved. The rate of eradication failure after treatment in *H. pylori*-positive children with upper gastrointestinal

Table I. Diagnostic Value of HpSA Test and HpIgG in Children with Upper Gastrointestinal Complaints, on the Basis of Agreement with UBT

	HpSA test	HpIgG
Sensitivity (%)	86	76.7
Specificity (%)	84.1	90.9
Positive predictive value (%)	84	89
Negative predictive value (%)	86	80
False-positive	15.9	9.1
False-negative	14	23.3

HpSA: *Helicobacter pylori* stool antigen. HpIgG: *Helicobacter pylori* immunoglobulin G. UBT: Urea breath test.

complaints who were compliant to treatment was 11.7%.

Discussion

H. pylori infection can be diagnosed by invasive techniques requiring endoscopy and biopsy or by noninvasive techniques. The gold standard is generally endoscopic biopsy-based testing, but it is difficult to apply such an invasive method in all children. In addition, endoscopic biopsy-based tests have disadvantages such as being invasive, expensive, or inapplicable for a widespread population[4]. Thus, several noninvasive methods have been widely used to diagnose *H. pylori* infection in children. These noninvasive tests are recommended as routine diagnostic tools in high-income countries but they are often not accessible in developing countries[11]. UBT is expensive, requires equipment, and is more difficult to perform than HpSA. HpSA is cheap, easy to perform, applicable for a widespread screen, and does not require equipment.

The UBT has high sensitivity and specificity rate (>95%) both in adults and children (2,12). This test has been accepted as a reliable test for *H. pylori* infection. However, it is technically more difficult to perform in young children and infants. Sensitivity of the UBT was not influenced by the age of the patient but specificity was lower, although not statistically different, in children <6 years of age (86%) versus in children >6 years (95%)[1]. Because of specificity concerns, we included children >6 years in our study.

The use of the HpSA test is being advocated as a safe and inexpensive test for diagnosing *H. pylori*. The advantages of the HpSA test include that it is noninvasive, specimens can be collected, transported, and preserved easily, test results are not affected by medication being taken by the patient, test results are clear, even to the naked eye, despite being an ELISA test, no invasive procedure such as endoscopy is required, and finally, it is cost-effective. Thus, the HpSA test is much easier to perform in children than any other test for *H. pylori*[13].

The accuracy of the HpSA test as a method for the primary diagnosis of *H. pylori* infection has been evaluated in many pediatric studies. Investigations have found a wide range of sensitivity and specificity, from 67% to 100% and from 61% to 100%, respectively[11]. Kalach et al.[14] evaluated a monoclonal enzyme stool antigen assay in 29 infected and 99 noninfected children, and they showed that the overall sensitivity, specificity, and positive and negative predictive values were 86.2%, 92.9%, 78.1%, and 95.8%, respectively, with an accuracy of 91.4%. Hino et al.[15]) evaluated a monoclonal enzyme stool antigen assay in 92 children with histology and rapid urease test as reference methods, and they found that the overall sensitivity, specificity, and positive and negative predictive values were 97.5%, 94.7%, 95.1%, and 97.3%, respectively. Nguyen et al[11]. evaluated the sensitivity and specificity of a monoclonal enzyme stool antigen assay for diagnosis of *H. pylori* infection in 232 children (age range: 3–15 years) who were positive for *H. pylori* infection by culture from biopsies. They found that the sensitivity of HpSA was 96.6% (95% confidence interval [CI] 93.3-98.5) and the

Table II. Comparison of the Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, False-Positives and False-Negatives of the HpSA Test and HpIgG after Eradication

	HpSA test	HpIgG
Sensitivity (%)	88.9	77.8
Specificity (%)	84	36
Positive predictive value (%)	66	30
Negative predictive value (%)	95	81
False-positive	16	64
False-negative	11.1	22.2

HpSA: *Helicobacter pylori* stool antigen. HpIgG: *Helicobacter pylori* immunoglobulin G. UBT: Urea breath test.

specificity was 94.9%. In the present study, the sensitivity, specificity, PPV, NPV, FP and FN values of the HpSA in children with upper gastrointestinal complaints were 86%, 84.1%, 84%, 86%, 15.9%, and 14%, respectively. HpSA test results may be affected by differences of antigenic diversity and serotypes of *H. pylori* in different countries. For this reason, there is discordance among the results of previous studies. In our study, diagnostic values of the HpSA test were the same as those reported in previous studies.

Therapeutic monitoring is as important as diagnosis in *H. pylori* infection, especially in children. Gościniak et al[16]. evaluated 62 children with chronic gastritis who were positive for *H. pylori* infection by cultures and histology of gastric biopsy specimens and a stool antigen test and 45 control subjects. They showed that 4-6 weeks after eradication therapy (clarithromycin, amoxicillin, omeprazole), the sensitivity, specificity, PPV, and NPV of the HpSA test were 88.9%, 96.2%, 80%, and 98%, respectively. In previous studies, the sensitivity and specificity of the HpSA after eradication therapy in children ranged from 88.9% to 100% and from 70% to 100%, respectively[7,17,18]. In accordance with these results, in this study, the sensitivity, specificity, PPV, NPV, FP, and FN values of the HpSA test, which was performed after eradication therapy, were 88.9%, 84%, 66%, 95%, 16%, and 11.1%, respectively. Based on these results, the monoclonal HpSA could be used in monitoring treatment and verifying eradication of *H. pylori* infection.

Regarding noninvasive tests, serum antibodies have the advantages of simplicity, low cost and utility for epidemiological studies and screening programs[19]. However, the serum HpIgG antibody has low sensitivity in young children because of an immature immune response to *H. pylori* in childhood[1]. Cherian et al[20]. observed satisfactory sensitivity, specificity, PPV, and NPV for HpIgG, as 57.9%, 77.4%, 92.0%, and 29.9%, respectively, in 149 children with *H. pylori* infection. The greatest disadvantage is that the clinicians are not able to distinguish between active infection and a previous contact. Antibody level persists in the blood for a long time after the treatment, so persistent antibodies will lead to false-positive results[10]. Hence, serology is not useful for monitoring treatment of *H. pylori* infection. In the present study, HpIgG sensitivity, specificity, PPV, NPV, FP, and FN values were detected to be 76.7%, 90.9%, 89%, 80%, 9.1%, and 23.3%, respectively, in children with upper gastrointestinal complaints. After eradication therapy, the overall HpIgG sensitivity, specificity, PPV, NPV, FP, and FN values were detected as 77.8%, 36%, 30%, 81%, 64%, and 22.2%, respectively. Thus, we showed that the serological test could not be used for diagnosis of *H. pylori* infections or for follow-up after eradication treatment in children.

Eradication therapy is recommended for children who have both known active *H. pylori* infection and gastrointestinal complaints. The optimum treatment regimen for *H. pylori* in children has not been determined. Triple treatment including a proton pump

inhibitor and clarithromycin, combined with either amoxicillin or metronidazole, has been recommended for children with *H. pylori* infection[1]. Effective therapy in adults is defined as successful eradication of infection in a minimum of 80% of treated subjects. It appears that treatment options that have been effective in adults will also be effective in children[2]. Testing to confirm eradication of infection and the resolution of associated symptoms is as important as diagnosis in *H. pylori* infection in children. Unsuccessful therapy often results from the patient's non-compliance with the medication regimen or from antimicrobial resistance[21]. In our study, the triple treatment regimen including a proton pump inhibitor and clarithromycin, combined with amoxicillin, was started in 43 *H. pylori*-positive children who had upper gastrointestinal complaints. Eradication was achieved in 73.5% and compliance to treatment was 85.3% in *H. pylori*-positive children with upper gastrointestinal complaints. Non-compliance to treatment was observed in 55.5% of children in whom *H. pylori* eradication could not be achieved. The rate of eradication failure after treatment in *H. pylori*- positive children with upper gastrointestinal complaints who were compliant with treatment was 11.7%. In our study, unsuccessful therapy resulting from the patient's non-compliance might have been associated with the low educational levels of parents.

In the present study, we wanted to first evaluate the diagnostic value of HpSA and serologic tests before and after eradication therapy for *H. pylori* in children with upper gastrointestinal complaints using the UBT test. We believe that the HpSA test can be used for both diagnosis of *H. pylori* infection and to determine the efficacy of eradication treatment in children. No other tests have similar advantages, because they either give false-positive results for a longer time or they are too expensive. In the present study, UBT was only performed in children with upper gastrointestinal complaints for ethical reasons, and this fact may have affected our results.

In conclusion, we demonstrated that the HpSA test is highly sensitive and specific for the diagnosis of *H. pylori* in children with upper gastrointestinal complaints living in Western Anatolia, Manisa region. The HpSA test for diagnosis of *H. pylori* infection is a useful, inexpensive, cheap, and easy to perform method. In addition, the HpSA test is a reliable noninvasive method for confirming eradication of *H. pylori* infection in children after treatment. In contrast to the HpSA test, serology is not a reliable method for the diagnosis of *H. pylori* infection or for confirming eradication in children with upper gastrointestinal complaints. However, there is a need for further studies with a larger number of patients for evaluation of these results.

REFERENCES

1. Veres G, Pehlivanoglu E. *Helicobacter pylori* infection in pediatrics. Helicobacter 2007; 12: 38-44.
2. Gold BD, Colletti RB, Abbott M, et al. *Helicobacter pylori* infection in children: recommendations for diagnosis and treatment. J Pediatr Gastroenterol Nutr 2000; 31: 490-497.
3. Crone J, Gold BD. *Helicobacter pylori* infection in pediatrics. Helicobacter 2004; 9: 49–56.
4. Ohkura R, Miwa H, Morai T, et al. Usefulness of a novel enzyme immunoassay for the detection of *Helicobacter pylori* in feces. Scand J Gastroenterol 2000; 35: 49–53.
5. Bode G, Rothenbacher D, Brenner H, Adler G. Variation in the 13C-urea breath test value by nationality in *Helicobacter pylori*-infected children. Scand J Gastroenterol 1998; 33: 468-472.
6. Kindermann A, Demmelmair H, Koletzko B, Krauss-Etschmann S, Wiebecke B, Koletzko S. Influence of age on 13C-urea breath test results in children. J Pediatr Gastroenterol Nutr 2000; 30: 85–91.
7. Yang HR, Seo JK. Helicobacter pylori stool antigen (HpSA) tests in children before and after eradication therapy: comparison of rapid immunochromatographic assay and HpSA ELISA. Dig Dis Sci 2008; 53: 2053-2058.
8. Weingart V, Rüssmann H, Koletzko S, et al. Sensitivity of a novel stool antigen test for detection of *Helicobacter pylori* in adult outpatients before and after eradication therapy. J Clin Microbiol 2004; 42: 1319-1321.
9. Malfertheiner P, Megraud F, O'Morain C, et al. Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III consensus report. Gut 2007; 56: 772–781.
10. Gatta L, Ricci C, Tampieri A, Vaira D. Non-invasive techniques for the diagnosis of *Helicobacter pylori* infection. Clin Microbiol Infect 2003; 9: 489-496.
11. Nguyen TV, Bengtsson C, Nguyen GK, Granström M. Evaluation of a novel monoclonal-based antigen-in-stool enzyme immunoassay (Premier Platinum HpSA PLUS) for diagnosis of *Helicobacter pylori* infection in Vietnamese children. Helicobacter 2008; 13: 269-273.
12. Rowland M, Lambert I, Gormally S, et al. Carbon 13-labeled urea breath test for the diagnosis of *Helicobacter pylori* infection in children. J Pediatr 1997; 131: 815–820.

13. Arikan S, Kocakusak A, Barut G, et al. Helicobacter pylori stool antigen test: results of a prospective study. Surg Today 2004; 34: 318–322.

14. Kalach N, Nguyen VB, Bergeret M, et al. Usefulness and influence of age of a novel rapid monoclonal enzyme immunoassay stool antigen for the diagnosis of *Helicobacter pylori* infection in children. Diagn Microbiol Infect Dis 2005; 52: 157-160.

15. Hino B, Eliakim R, Levine A, et al. Comparison of invasive and non-invasive tests diagnosis and monitoring of *Helicobacter pylori* infection in children. J Pediatr Gastroenterol Nutr 2004; 39: 519-523.

16. Gościniak G, Przondo-Mordarska A, Iwańczak B, Blitek A. *Helicobacter pylori* antigens in stool specimens of gastritis children before and after treatment. J Pediatr Gastroenterol Nutr 2003; 36: 376-380.

17. Roggero P, Bonfiglio A, Luzzani S, et al. *Helicobacter pylori* stool antigen test: a method to confirm eradication in children. J Pediatr 2002; 140: 775-777.

18. Oderda G, Rapa A, Marinello D, Ronchi B, Zavallone A. Usefulness of *Helicobacter pylori* stool antigen test to monitor response to eradication treatment in children. Aliment Pharmacol Ther 2001; 15: 203–206.

19. Chang MC, Wu MS, Wang HH, Wang HP, Lin JT. Helicobacter pylori stool antigen (HpSA) test: a simple, accurate and non-invasive test for detection of *Helicobacter pylori* infection. Hepatogastroenterology 1999; 46: 299-302.

20. Cherian S, Burgner DP, Carson CF, et al. Diagnosis of *Helicobacter pylori* infection in a high-prevalence pediatric population: a comparison of 2 fecal antigen testing methods and serology. Pediatr Gastroenterol Nutr 2008; 47: 130-135.

21. Romano M, Iovene MR, Russo MI, et al. Failure of first-line eradication treatment significantly increases prevalence of antimicrobial-resistant Helicobacter pylori clinical isolates. J Clin Pathol 2008; 61: 1112-1115.