Detection of *Helicobacter pylori* antigen in stool samples for diagnosis of infection in children

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اختبار الكشف عن مستضدات الملويَّة البوابيَّة في عينات البراز لتشخيص العدوى لدى الأطفال

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الخلاصة: قام الباحثان بإجراء هذه الدراسة لتقييم اختبار مستضدي غير باضع يجرى على عينات البراز، لتشخيص العدوى بجراثيم الملويَّة البوابيَّة في الأطفال، مقارنةً بالاختبار الهستوباثولوجي الذي يجرى على الخزعات المعوية (المعيار الذهبي). وشملت الدراسة 96 طفلاً تراوحت أعمارهم بين عام واحد وخمسة أعوام، ممن كانوا يعانون من أعراض عسر الهضم وأحيلوا لإجراء تنظير داخلي في مستشفى الأطفال في تبريز، بجمهورية إيران الإسلامية، في الفترة من أيار/مايو 2003 وحتى آذار/مارس 2004. وجاءت نتائج الاختبار المستضدي في عينات البراز إيجابية لدى 34 طفلاً من بين 62 طفلاً أثبت اختبار النسيج المعوي إيجابيتهم لعدوى الملوية البوابية، كما جاءت نتائج اختبار عينة البراز سلبية لدى 27 من بين 34 مريضاً ممن كانت نتائج اختبار النسيج لديهم سلبية. وأظهرت النتائج انخفاض معدلات الحساسية والنوعية في هذا الاختبار (54.8٪ و79.4٪ على التوالي)، بالمقارنة بالمعيار الذهبي. كما بلغت القيمة التكهّنية السلبية 82.9٪ و49.9٪. ومع ذلك فإن استخدام هذا الاختبار قد يكون مفيداً في مجال التحري الجموعي لجرثومة الملوية البوابية.

ABSTRACT The study evaluated a non-invasive antigen test of stool samples for the diagnosis of *Helicobacter pylori* infection in children compared with histopathology of gastric biopsies (gold standard). The study included 96 children aged 1–15 years old with dyspeptic symptoms referred for endoscopy at Tabriz Children's Hospital, Tabriz, Islamic Republic of Iran from May 2003 to March 2004. Of 62 children who were positive by histology, 34 were *H. pylori* stool antigen positive and of 34 patients with negative histology, 27 had negative stool test. The sensitivity and specificity of the test were low (54.8% and 79.4% respectively) compared with the gold standard and the positive and negative predictive values were 82.9% and 49.9%. However, the test may be useful for mass screening for *H. pylori*.

Détection de l'antigène fécal d'*Helicobacter pylori* pour le diagnostic de l'infection chez l'enfant

RÉSUMÉ Cette étude a évalué un test non invasif de détection de l'antigène fécal d'*Helicobacter pylori* (HpSA - pour *Helicobacter pylori stool antigen*) chez l'enfant *versus* l'histopathologie de biopsies gastriques (la norme en l'espèce). Ont été inclus dans l'étude 96 enfants âgés de 1 à 15 ans adressés entre mai 2003 et mars 2004 à l'hôpital pour enfants de Tabriz, en République islamique d'Iran, pour endoscopie sur présentation de symptômes dyspeptiques. Sur les 62 enfants positifs à l'histologie, 34 se sont avérés HpSA-positifs tandis que sur les 34 patients présentant une histologie négative 27 étaient HpSA-négatifs. Comparativement au test de référence, ce test antigénique a fait preuve d'une sensibilité et d'une spécificité peu convaincantes, à savoir respectivement 54,8 % et 79,4 %, les valeurs prédictives positive et négative étant quant à elles de 82,9 % et 49,9 %. Toutefois, ce test peut avoir son utilité dans le cadre d'un dépistage de masse d'*Helicobacter pylori*.

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Introduction

Gastric and ulcer peptic disease is a common disease in the community, especially in children. Considering the close relationship between peptic ulcer and gastritis caused by *Helicobacter pylori*, the accurate and early diagnosis of infection by this micro-organism is very important so as to provide prompt and convenient treatment to the affected children [*1,2*]. The diagnostic tests for *H. pylori* infection are principally of 2 types: invasive and non-invasive. Invasive diagnostic methods include polymerase chain reaction (PCR), rapid urease test (RUT), endoscopy of the upper digestive tract and biopsy of the gastric mucosa for pathological examination. Non-invasive diagnostic methods include serologic enzyme-linked immunosorbent assay (ELISA), urea breath test (UBT) with C13 or C14 labelled urea and the *H. pylori* stool antigen test (HpSA) [*1–4*].

No study is available from the East Azerbaijan area or elsewhere in the Islamic Republic of Iran to compare the results of invasive and non-invasive diagnostic methods for detecting of *H. pylori* antigen in stool samples in children in the community. This research study was therefore carried out to ascertain whether detection of *H. pylori* antigen in the stool sample using immunoassay can substitute for diagnostic tests such as endoscopy and biopsy of the gastric mucosa, which, although standardized, are painful procedures that may involve a high level of risk, especially in children.

Methods

The study design was observational, descriptive with convenience sampling.

Sample

The study was carried out on all children aged from 1–15 years old with dyspeptic symptoms who were referred to the endoscopic section of Tabriz children's hospital during an 11-month period from May 2003 to March 2004. The children were included if they suffered dyspepsia, chronic gastric pain (gastric pain for more than 3 months with at least one course of pain each month), recurrent anorexia, vomiting and heartburn, bleeding in the upper or lower digestive tract and recurrent diarrhoea. Patients with severe diarrhoea and those who had received antibiotics or proton pump inhibitors as well as antacids in the previous 4 weeks were excluded from the study.

The children and their parents were informed about the necessary clinical and laboratory examination procedures and their consent was taken before the start of the study.

Data collection

Endoscopy of the digestive system was performed by video endoscopy (Olympus video-gastroscope Evis 100 type XP 20). A total of 3 biopsy samples were taken from observation areas and sent to the pathology laboratory: 2 from the antrum at 2 cm intervals and 1 from the gastric corpus. Histological preparations were done under haematoxylin–eosin stain and also by modified May–Grunwald–Giemsa staining procedure. The presence of *H. pylori* organisms was reported in 6 groups ranging from grade 0 to grade 5 using the modified scoring system (MSS) criteria for assessing histopathology samples [*5*].

Stool samples for the HpSA test were taken from patients and sent to the microbiology laboratory. Detection of *H. pylori*

antigens in stool was carried out by ELISA polyclonal antibody test (Equipar HpSA test, Saronno, Italy).

Analysis

After gathering simple frequencies and percentages, the data were transferred to computer and analysed by *SPSS* using McNamara test and the chi-squared test. Sensitivity, specificity and positive and negative predictive values were calculated using the histopathology results as the gold standard.

Results

The study group was 96 children aged from 1–15 years old with a mean age of 8.3 years (standard deviation 0.3 years); 70 (72.9%) were outpatients and 26 (27.1%) were admitted as inpatients in the gastroenterology division or other divisions of the children's hospital. There were 65 males (67.7%) and 31 females (32.3%).

Table 1 shows the results of histopathology examination of biopsy specimens from the gastric areas. The results of the HpSA antigen test in stool samples of patients are shown in Table 2.

Comparing the incidence of *H. pylori* cases from biopsy and stool testing, it was found that out of 62 (64.6%) patients reported positive for *H. pylori* in histopathology of biopsy samples (the gold standard), only 34 cases were *H. pylori* positive by the HpSA test (Table 3). According to this study, the sensitivity, specificity, positive and negative predictive values for the HpSA test were 54.8%, 79.4%, 82.9% and 49.9% respectively. There was a significant difference between the 2 groups who were positive for *H. pylori* in biopsy sample and negative for *H. pylori* in biopsy in terms of *H. pylori* variance in the HpSA test ($P < 0.001$, McNamara test).

Table 4 presents the *H. pylori* histopathology grading in biopsy samples based on MSS criteria and the HpSA positive cases. It was observed that as the grade of *H. pylori* in biopsy samples increased, the possibility of the stool sample being positive increased too ($P < 0.001$). Out of 31 patients whose histological examination indicated

Table 1 **Frequency of children positive by histopathology of gastric biopsy, based on modified scoring system (MSS) criteria**

Biopsy grades	No. of children	%	Total % (MSS criteria)
0	34	35.4	35.4
1	22	22.9	58.3
2 & 3	31	32.3	90.6
4 & 5	9	9.4	100.0
Total	96	100.0	

Table 2 **Frequency of children positive by *Helicobacter pylori* stool antigen (HpSA) test, based on modified scoring system (MSS) criteria**

Test	No. of children	%	Total % (MSS criteria)
HpSA negative	55	57.3	57.3
HpSA positive	41	42.7	100.0
Total	96	100.0	

Table 3 **Comparison of frequency of children positive by histopathology of gastric biopsy (gold standard) and *Helicobacter pylori* stool antigen (HpSA) test**

Test	Biopsy negative No.	Biopsy positive No.	Total No.
HpSA negative	27	28	55
HpSA positive	7	34	41
Total	34	62	96

Table 4 **Comparison of frequency of children positive by histopathology of gastric biopsy (grades based on modified scoring system criteria) and *Helicobacter pylori* stool antigen (HpSA) test**

Test	Biopsy grades				
	0 No.	1 No.	2 & 3 No.	4 & 5 No.	Total No.
HpSA negative	27	14	14	0	55
HpSA positive	7	8	17	9	41
Total	34	22	31	9	96

grade 2 or 3, less than of half cases ($n = 14$) were *H. pylori* negative in stool samples. The most important finding was that all of 9 patients determined as grade 4 or 5 from biopsy samples were also *H. pylori* positive in stool samples (Table 4). In addition, out of all 34 cases of grade 0 of *H. pylori* in biopsy sample, 27 cases were reported *H. pylori* negative.

Discussion

To determine *H. pylori* infection in humans, multiple laboratory methods have been reported [*5–8*]; however, based on estimated resources, the histological examination of biopsy samples is considered as the gold standard among invasive diagnostic methods to diagnose this micro-organism with a sensitivity and specificity of 100% [*2*].

In comparison to biopsy, cultivation of bacteria requires specific environmental conditions and a fairly long time for the organism to grow, which are considered as the shortcomings of the biopsy cultures, although the rate of success is reported to be nearly 100% when there is accuracy in the cultivation of biopsy samples [*2*]. Other invasive procedures, the RUT and antibody measurements in blood using ELISA are less sensitive and specific in children as compared with adults [*1,2*].

The UBT is a non-invasive method that possesses more accuracy to diagnose infectious from non-infectious cases in children and adults and is reported to be 100% sensitive and 92% specific in diagnosing *H. pylori* infection in children. However, the test is difficult to perform in children and require the child's collaboration [*2*].

According to a study in Taiwan, 53 children affected by dyspepsia were assayed [*6*]. The diagnostic accuracy of different tests was as follows: culture 98.1%, RUT 96.2%, pathology 98.1%, PCR 94.3%, serology 84.9%, UBT 100% and HpSA 96.2%. According to their study, all the above-mentioned methods, except serology, are considered valuable diagnostic methods in the diagnosis of *H. pylori* in children [*6*]. This and similar studies in Germany, China, France, England, Spain, Italy and Poland revealed that non-invasive methods such as HpSA possess high sensitivity and specificity as compared with other diagnostic methods in children such as UBT [*7–15*].

In another study in England, the results of detection of HpSA were compared in 72 patients suffering from dyspepsia using 3 stool antigen enzyme immunoassay kits versus endoscopy biopsy samples [*16*]. The sensitivity of the Premier Platinum Hp SA kit (Meridian Diagnostics, Cincinnati, Ohio, USA) was 63.6% and specificity 92.6%, the sensitivity of FemtoLab Cnx kit (Dako, Ely, Cambridgeshire, UK) was 88% and specificity 97.6%, and the sensitivity of the Hp Ag kit (Dia.Pro, Milan, Italy) was 56% and specificity 97.6% [*16*].

Our study was an HpSA test using polyclonal antibody detected by ELISA and the sensitivity and specificity were 54.8% and 79.4% respectively. Our results are compatible with the above study [*16*] but not with the results of the study in

Taiwan [*6*]. Despite minor difficulties such as the collection of a new stool sample, the performance of HpSA is an alternative and reliable substitute for the UBT test as a non-invasive diagnostic method in children. Secondly, the test is not expensive and does not require a blood sample.

Considering the sensitivity and specificity of the HpSA test in our study, the practical value of this test seems to lie more in eliminating cases that are negative for *H. pylori* and accordingly this test could be applied in mass screening such as at regional school level.

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