Application of Stool-PCR test for diagnosis of Helicobacter pylori infection in children

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AIM: To evaluate the usefulness of stool-PCR test for diagnosis of Helicobacter pylori (H pylori) infection in pediatric populations.

METHODS: Based on endoscopic features (including nodular gastritis, erosive duodenitis and ulcer) and/or a positive rapid urease test (RUT) obtained during endoscopy, 28 children from a group of children admitted to the Children's Medical Center of Tehran for persistent upper gastrointestinal problems were selected to compare biopsy-based tests with stool-PCR. Their gastric activity and bacterial density were graded by the updated Sydney system, and their first stool after endoscopy was stored at -70°C. Biopsies were cultured on modified campy-blood agar plates and identified by gram-staining, biochemical tests, and PCR. Two methods of phenol-chloroform and boiling were used for DNA extraction from H pylori isolates. Isolation of DNA from stool was performed using a stool DNA extraction kit (Bioneer Inc, Korea). PCR was performed using primers for detection of vacA, cagA, and 16s rRNA genes in both isolates and stool.

RESULTS: Sixteen out of 28 child patients (57%) were classified as H pylori positive by biopsy-based tests, of which 11 (39%) were also positive by stool-PCR. Sensitivity and specificity of stool-PCR was 62.5% and 92.3% respectively. H pylori was observed in histological sections for 10 out of 11 stool-positive patients. Association was observed between higher score of H pylori in histology and positivity of stool-PCR. Also association was observed between the more severe form of gastritis and a positive stool-PCR.

CONCLUSION: Association between higher score of H pylori in histology and a positive stool-PCR make it a very useful test for detection of H pylori active infection in children. We also suggest that a simple stool-PCR method can be a useful test for detection of H pylori virulence genes in stool.

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some disadvantages for infants and very young children, as well as patients with certain neurological disorders[8,9]. 

*H pylori* is not an intestinal pathogen, and therefore is expected to be present in low concentrations in stool; however, it can be detected in stool specimens by *H pylori* stool-antigen (HpSA) test, PCR, or even culture[6-12]. The HpSA test has been shown to be very useful, especially in children; however, various commercial tests have shown some discrepancies in different geographical areas[13-15]. Stool-culture is a very specific method; however, the massive numbers of diverse micro-organisms in stool makes it very difficult in routine practice[16,17]. Stool-PCR may also be a very useful method in detection of *H pylori* infection, but reported success rates for the detection of *H pylori* DNA in feces vary from 25% to 100%/8,18. This variability is probably due to *H pylori* degradation in the gastrointestinal tract and/or the presence of inhibitors such as complex polysaccharides1637. The purpose of this study was to evaluate the usefulness of the stool-PCR test for diagnosis of *H pylori* infection in pediatric populations.

**MATERIALS AND METHODS**

**Patients**

Based on endoscopic features (including nodular gastritis, erosive duodenitis, or ulcers) and/or a positive rapid urease test obtained during endoscopy, 28 children from a group of children admitted to a children’s medical center in Tehran for persistent upper gastrointestinal problems were selected to compare biopsy-based tests and stool-PCR. Of these patients, two antral biopsies similar to that of RUT were obtained for culture and histology, and the 16手动reduction tests. The H pylori status

Sixteen out of 28 child patients (57%) were classified as *H pylori* positive by biopsy-based tests. Of 16 *H pylori* positive children 6 were positive by culture, 5 were positive by all of the 3 tests, and 5 were positive by RUT plus histology.

**PCR results**

DNA isolated from culture positive controls showed amplification for *H pylori* specific primer(s) including vacA (s, m), cagA, and 16SrRNA. Stool-PCR positive controls, which were 3 uninfected feces from the *H pylori*-negative patient containing known concentrations of 26695 *H pylori* ATCC strain, showed amplification for *H pylori* DNA only after purification by column chromatography procedure. No amplification was observed for the negative stool-PCR controls (stool

**DNA extraction and PCR**

Two methods of phenol-chloroform and boiling were used for DNA extraction from *H pylori* isolates. For the first one, a pool of colonies in 2 mL sterile 0.9% NaCl, was centrifuged at 10000 g, the pellet was resuspended in 400 μL of extraction buffer (10 mmol/L Tris-HCl, pH 8.0, 5 mmol/L EDTA, 0.1% sodium dodecyl sulfate), and proteinase K at final concentration of 0.5 mg/mL was added to homogenizes. Samples were incubated at 55°C for 2-4 h before incubation at 95°C for 10 min. DNA was purified by phenol-chloroform, precipitated by absolute ethanol at -20°C in presence of 0.3 mol/L sodium acetate, pelleted by centrifugation at 12000 g for 30 min and allowed to dry in air. The pellet in sterile double-distilled water was quantified by measuring the optical density at 260 nm and stored at -20°C until they were used as PCR templates. For the second method, a loopful of colonies was suspended in 1 mL of phosphate buffer saline (PBS, pH 7.6), washed by centrifugation at 14000 g for 2 min, and resuspended in 50 μL of sterile, double distilled water. Tubes were then boiled at 95°C for five minutes and 2 μL of 1/5 dilution of this extract (containing approximately 20 ng of DNA) was immediately used as template for PCR. Isolation of DNA from stool was performed using a stool DNA extraction kit (Bioneer Inc, Korea), where substances inhibiting PCR were removed by filtration according to the manufacturer’s instructions. Stool-PCR controls were 3 uninfected feces from the *H pylori*-negative patient (as determined by endoscopy-based tests) seeded or not seeded with known concentrations (equivalent to McFarland No. 5) of 26695 *H pylori* ATCC strain.

PCR primers (Faza Biotech Inc, Iran) were designed on the basis of published sequences of *H pylori* 16S rRNA, vacA, and cagA[8,21]. Table 1 resumes the sequences and experimental details for PCR.

**RESULTS**

**The H pylori status**

Sixteen out of 28 child patients (57%) were classified as *H pylori* positive by biopsy-based tests. Of 16 *H pylori* positive children 6 were positive by culture, 5 were positive by all of the 3 tests, and 5 were positive by RUT plus histology.

**Biopsy-based tests**

Culture of biopsy samples was performed as previously described8,12,18. Briefly, antral biopsies were placed in a modified campy-thio medium and incubated at 37°C under a micro-aerobic atmosphere. After 3 d, 20 μL of the enrichment culture was streaked onto modified campy-blood agar and incubated for 5-10 d until colonies were evident. The grown colonies were identified by gram-staining, oxidase, urease, and nitrate-reduction tests.

RUT was performed using urea broth as previously described. The RUT result was read either within 2 h at endoscopy room or after overnight incubation under a micro-aerobic atmosphere at 37°C according to the previously described protocol8,12,18. Histological examination of the biopsies was performed after H&E, and Geimsa staining *H pylori* density, gastritis, and inflammation were graded according to the modified Sydney system19,20. The cases of gastritis with follicular formation were classified as follicular gastritis either with or without activity20.
specimens from *H. pylori*-negative patients), even after purification procedure. Eleven biopsied children showed positive stool-PCR of which 10 were positive by biopsy-based tests (Table 2). Sensitivity and specificity of stool-PCR were 62.5% and 92.3% respectively.

In this work, detection of *H. pylori* specific virulence genes in both isolates and stool (Table 3) was compared. Also, association between endoscopic features, pathology, score of bacteria, and a positive stool-PCR was studied (Table 4). *H. pylori* was observed in histological sections of 10 out of 11 stool-positive patients and association was observed between higher score of *H. pylori* in histology and a positive stool-PCR.

**DISCUSSION**

In our previous study,[12] we successfully cultured *H pylori* from stool; however, the sensitivity of stool-culture was low. Using PCR, we detected *H pylori* specific genes in isolates and stool in sick and healthy children. However, when fecal extracts were not subjected to column chromatography, there were no results even for the positive controls. This suggests that the method of DNA extraction used in this work efficiently removed the DNA from stool; however, the sensitivity of stool-culture was 50%. However, when fecal extracts were not subjected to column chromatography, there were no results even for the positive controls. This suggests that the method of DNA extraction used in this work efficiently removed the DNA from stool; however, the sensitivity of stool-culture was 50%.
In this work, by detection of various *H. pylori* specific genes in stools, 62.5% sensitivity and 92.3% specificity was observed for stool-PCR (Table 2). Nevertheless, by PCR only one or two out of three *H. pylori* specific genes were detectable (Table 3). While this permits us to think that the absence of amplification is related to the absence of the detecting gene from the genome or the absence of intact template DNA (in stool), it would be a premature conclusion, since PCR-based absence of an ORF does not necessarily mean its absence from the genome. Also, in a highly recombinating genome like *H. pylori*, PCR primer annealing sites can pose problems and amplifications may not be generated\cite{8,10-11,14,22-24}. Thus, we think that for genotyping of *H. pylori* from stool, using more than one primer for each gene may enhance detection rate. Many investigators have proposed semi-nested or nested PCR as more sensitive methods for stool-PCR\cite{8,10}. Although these methods reduce background, their disadvantages would be presence of false positive results due to detection of dead bacteria in stool even in low amounts. Sensitivity and specificity of stool-PCR method in this work were acceptable, suggesting that PCR method used in this work was quite adequate for this evaluation.

*H. pylori* is not an intestinal pathogen, and therefore is expected to be present in low concentrations in stool; however, the status of the infection of *H. pylori* may influence its density in stool. Thus, we compared histological scoring of *H. pylori* with pathological grading and also with the results of stool-PCR. Concordance was observed between higher score of *H. pylori* in histological sections and a positive stool-PCR (Table 4). Also, association was observed between the more severe form of gastritis and a positive stool-PCR. Therefore, the degree of stomach colonization by *H. pylori* may be important for successful detection of DNA in stool samples. Otherwise, the amount of bacteria excreted in stool may reveal information on the status of *H. pylori* infection. Consequently, the association between a higher score of *H. pylori* in histology and a positive stool-PCR make it a very useful test for detection of pediatric *H. pylori* infection.

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**COMMENTS**

**Background**

A reliable non-invasive test for detection of *Helicobacter pylori* (*H. pylori*) infection in routine practice is essential, especially for children since the application of biopsy-based tests is more difficult for them. Serological tests do not necessarily indicate active infection by *H. pylori*, and urea breath test (UBT) is expensive and not available in routine clinical laboratories, especially in developing countries. The *H. pylori* stool-antigen (HpSA) test has been shown to be very useful, especially in children; however, various commercial tests have shown some discrepancies in different geographical areas. Stool-PCR may be a very useful test in specific detection of *H. pylori*. In this study, we evaluated the performance of stool-PCR in diagnosis of active infection in children.
Research frontiers
Stool-PCR is a very useful method for detection of H pylori genes in stool. It is interesting because H pylori specific genes, including virulence genes and the genes involved in its resistance to antibiotics, can be detected by this method. Furthermore, a positive stool-PCR has significance in relation to the status of stomach colonization by H pylori.

Innovations and breakthroughs
A stool-PCR method such that used in this work may represent a very specific test for diagnosis of H pylori infection. This is the first study to report association between a positive stool-PCR and the degree of stomach colonization, manifested by higher score of H pylori in histology.

Applications
A simple PCR method such that used in this work will be quite adequate for detection of H pylori infection.

Peer review
In this study, Falsafi et al. evaluated the performance of stool-PCR test for diagnosis of current H pylori infection in children. The content of the article can be interesting for gastroenterologists who work with the pediatric population, especially with very young children and patients with certain neurological disorders. Stool-PCR may be a very useful method in detection of H pylori infection.

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