Comparison of biopsy-based methods for the detection of *Helicobacter pylori* infection

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ABSTRACT

Various biopsy-based methods for the detection of *Helicobacter pylori* are evaluated to determine their sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), followed by polymerase chain reaction (PCR) for the 16S ribosomal RNA (rRNA) gene of *H. pylori* (16S PCR) to confirm the results. Seventy-five patients (65% [49] males, age range: 17–77 years, mean 42±14.6 years) with dyspeptic symptoms are included in the study. Gastric antrum biopsy specimens collected during endoscopy are tested using a urea agar base enriched with 40% urea solution (eUAB, Oxoid)), a commercial rapid urease test (Pronto Dry, Medical Instrument Corp, Switzerland), histopathology and 16S PCR. The eUAB test showed 97% sensitivity, 86% specificity, 84% PPV, 97% NPV and 91% accuracy when the diagnosis of *H. pylori* infection was made with positive Pronto Dry and histopathology. Pronto Dry showed 100% sensitivity, 82% specificity, 80% PPV, 100% NPV and 89% accuracy when the diagnosis of *H. pylori* infection was made on positive histopathology and eUAB. Thus, the eUAB can be used as a rapid urease test. It is economical and has a sensitivity and specificity comparable to a commercially available rapid urease test to detect urease activity of *H. pylori* in gastric biopsy.

KEY WORDS: Endoscopy, digestive system.
Helicobacter pylori.
Histology.
Polymerase chain reaction.
Urease.

Introduction

Helicobacter pylori infection occurs worldwide, colonising the stomachs of half of all humans.[1] It results in gastritis, peptic ulcer disease, mucosa-associated lymphomas and gastric carcinomas.[2–3] Prevalence varies from country to country, with existing evidence suggesting that the diversity in disease outcome may be attributed to variations in infecting strains.[3]

There are several methods for detecting *H. pylori* infection such as serology, gastric biopsy for culture, histological examination, assays for urease activity and molecular typing by polymerase chain reaction (PCR) amplification methods for the identification of *H. pylori*-specific genes.[4,5]

Urease produced by *H. pylori* catalyses the hydrolysis of urea to yield a high local concentration of ammonia that serves as the basis of diagnostic tests for the presence of *H. pylori* in the urease biopsy test and urea breath test. Urease activity of *H. pylori* is indicated by a change of colour from yellow to pink in the test.

Rapid urease tests are used widely during endoscopy to determine the presence or absence of *H. pylori*. In Pakistan, two commonly used rapid urease tests are the Pronto Dry (Medical Instrument Corp., Switzerland) and the CLO test (Delta West, Perth, Australia). Pronto Dry has the advantage of providing rapid results, can be stored at room temperature for two years and is simple to use at room temperature.[6,7]

In Pakistan, as in other developing countries, a limited amount of its gross national income is spent on healthcare services, with medical treatment largely paid for by the individual. Furthermore, there is a general trend towards keeping hospital charges to a minimum, in order to limit the movement of patients to competitors in the healthcare provider market. A number of rapid urease tests are available to test for *H. pylori* infection and there is ongoing demand for a cheaper, efficient urease test.

This study aims to evaluate the effectiveness of enriched urea agar base (eUAB), Pronto Dry and histopathology in the detection of *H. pylori* infection in the endoscopy suite of a hospital in a developing country and to compare sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy using a PCR method for the 16S ribosomal RNA (rRNA) gene of *H. pylori* (16S PCR) to confirm the diagnosis. The study uses 16S PCR because of its higher diagnostic yield compared to histopathology.[8] However, in view of the 60% seroprevalence of *H. pylori* in the population studied, PCR is not cost-effective as a diagnostic tool for *H. pylori* infection in this setting.[9]

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Materials and methods

Patients

Seventy-five patients with dyspeptic symptoms attending for endoscopy between February 2005 and January 2006 were enrolled in the study. The clinical symptoms at

presentation, diagnosis, drug treatment dosage and duration were noted, together with the endoscopy findings. Patients previously treated for *H. pylori* infection or who had received antibiotics, proton pump inhibitors (PPI), histamine-2 receptor blocker (H2RB) or bismuth compounds in the preceding four weeks were excluded.

Informed consent was obtained for oesophago-gastroduodenoscopy and additional biopsy in all the patients. The study was approved by the Ethics Review Committee of Aga Khan University Hospital. Endoscopy was performed after an eight-hour fast. All patients received sedation with intravenous midazolam and topical pharyngeal anaesthetic spray.

Sterilised biopsy forceps were used to obtain gastric biopsy specimens from an area of inflammation in the antrum. Two biopsy specimens each were removed for eUAB, Pronto Dry, histopathology and PCR. Biopsy specimens were removed from the biopsy forceps with a sterile needle, and were inserted in Pronto Dry and eUAB, while specimens for histopathology and 16S PCR testing were dispatched to the laboratory in formalin and 0.9% normal saline, respectively.

Urease test

Urea agar base enriched with 40% urea solution (Oxoid) is designed to detect urease activity in Enterobacteriaceae.[10] When inoculated with an organism which produces urease (e.g., *Proteus* spp.), urea is broken down to form ammonia. This raises the pH of the medium, which contains phenol red, and produces a red colour. It is not marketed to detect urease activity in gastric biopsy tissue; however, this study tested the feasibility of its use as a rapid urease test.

Briefly, 2.4 g urea agar base was suspended in 95 mL distilled water and dissolved completely by bringing to the boil. It was sterilised by autoclaving at 115˚C for 20 min. It was cooled to 50˚C and 5 mL sterile 40% urea solution was added aseptically. Urea agar base enriched with 40% urea, a light yellow solution, was dispensed in sterile Eppendorf tubes in 300-µL amounts and was allowed to set in the slope position and refrigerated at 2–8˚C prior to use.

Pronto Dry and eUAB results were read after 30 min and 1 h, respectively, as directed by the manufacturer. Colour change from yellow to pink was considered positive for both eUAB and Pronto Dry.

Histopathology

Haematoxylin and eosin (H&E)-stained tissue sections were used to detect the presence of *H. pylori* and to assess the degree of gastritis. In equivocal cases, Giemsa staining was used to confirm the presence of *H. pylori*.[11]

Polymerase chain reaction

Extraction of DNA from gastric tissue was performed as described previously.[12] Briefly, tissue was homogenised in 500 µL sterile water and centrifuged at 12,000 x*g* for 3 min. Then, 500 µL lysis buffer (100 mmol/L NaCl, 10 mmol/L Tris-HCl [pH 8.0], 25 mmol/L EDTA, 0.5% sodium dodecyl sulphate) and 10 µL proteinase K (10 mg/mL) were added. Incubation was carried out at 50˚C for 20 h, followed by phenol-chloroform extraction and ethanol precipitation.

The resulting pellet was allowed to dissolve in 40 µL TE buffer (10 mmol/L Tris-HCl [pH 7.4] and 0.1 mmol/L EDTA [pH 8.0]) for 20 h at 37˚C. Samples were stored at –20˚C prior to PCR amplification. DNA content and purity was determined by measuring the absorbance at 260 nm and 280 nm using a spectrophotometer (Beckman DU-600, USA).

Amplification

The PCR method was performed using extracted DNA as the template and *H. pylori*-specific 16S rRNA gene primers C97 5′-GCT ATG ACG GGT ATC C-3′ (276–291 forward) and C98 C 98, 5′-GAT TTT ACC CCT ACA CCA-3′ (681–698 reverse) to amplify a 16S rRNA amplicon of approximately 400 bp, as described previously.[13] Briefly, amplification was carried out in a total volume of 50 µL containing 2 µL of 2 mmol/L dNTPs, 1 µL containing 25 pmol primer C97, 1 µL containing 25 pmol primer C 98 (synthesised by an MGW automatic synthesiser), 2 units *Thermus aquaticus* (*Taq*) DNA polymerase (Promega, USA), 5 µL of 10x PCR reaction buffer (10 mmol/L Tris-HCl [pH 8.3], 50 mmol/L KCL, 0.1 % Triton X-100 [after dilution]), 3 mmol/L $MgCl_2$ and 1 µL DNA template containing 0.5 ng extracted DNA. The total volume was made up to 50 µL by double distilled water.

The reaction was carried out in a Perkin Elmer 480 thermal cycler. The amplification cycle consisted of initial denaturation at 94˚C for 5 min and then 94˚C for 1 min, primer annealing at 55˚C for 1 min and an extension at 72˚C for 90 sec. The final cycle included an extension step of 5 min at 72˚C to ensure full extension of the product. Samples were amplified through 35 consecutive cycles.

H. pylori ATCC 49503 genomic DNA was used as a positive control and double distilled water was used for negative control reactions with each batch of amplifications. The amplified PCR product (8 µL) was analysed on a 2% (w/v) agarose gel, stained with ethidium bromide and observed under ultraviolet light.

Sensitivity, specificity, PPV, NPV and accuracy of eUAB, Pronto Dry and histopathology were compared against 16S PCR. As PCR is very sensitive and not feasible to use routinely for the diagnosis of *H. pylori* infection, the sensitivity, specificity, PPV, NPV and accuracy of eUAB against positive Pronto Dry and histopathology were determined, while those for Pronto Dry were compared against positive eUAB and histopathology.

A diagnosis of *H. pylori* infection was made when 16S PCR or Pronto Dry and histology or eUAB and histology were positive. *H. pylori* was deemed to be absent when 16S PCR or Pronto Dry and histopathology were negative.

Statistical assessment

The Statistical Package for Social Science (SPSS, release 10.0.5) was used for data analysis. Descriptive analysis was used for demographic and clinical features. Results are presented as mean ± standard deviation (SD) for quantitative variables and as a number (percentage) for qualitative variables. Sensitivity, specificity, PPV and NPV were determined for eUAB, Pronto Dry and histopathology.

Results

In the 75 patients studied, presenting symptoms were abdominal pain in 43 (57%), heartburn in 15 (20%), bloating in 10 (13%) and vomiting in seven (10%). The main endoscopic findings were gastritis in 43 (57%),

Table 1. Comparison of various tests for *Helicobacter pylori* infection.

Diagnostic test	Sensitivity	Specificity	PPV	NPV	Accuracy
eUAB urease test *vs.* 16S PCR (n=75)	53%	78%	95%	18%	56%
Pronto Dry *vs.* 16S PCR (n=75)	57%	78%	95%	19%	59%
Histopathology *vs.* 16S PCR (n=75)	68%	78%	96%	25%	69%
eUAB urease test *vs.* Pronto Dry and histopathology (n=75)	97%	86%	84%	97%	91%
Pronto Dry *vs.* eUAB and histopathology (n=75)	100%	86%	84%	97%	91%

gastroesophageal reflux disease in 23 (31%), duodenitis in six (8 %) and duodenal ulcer in three (4%).

16S PCR was positive in 66 (88%) patients and negative in nine (12%). eUAB was positive in 37 (49%) and negative in 38 (51%). Pronto Dry was positive in 39 (49%) and negative in 36 (51%). Histopathology was positive in 47 (63%) and negative in 28 (37%). Sensitivity, specificity, PPV, NPV and accuracy of each test compared to 16 S PCR was low (Table 1).

Comparisons of eUAB with Pronto Dry and histopathology, and of Pronto Dry with eUAB and histopathology are shown in Table 1. Reaction time to positive was faster with Pronto Dry than with the eUAB test (94 % versus 74%, respectively, by 30 min; 100% versus 90%, respectively, by 60 min).

Discussion

The rapid urease test is used frequently for the diagnosis of *H. pylori* infection in routine gastrointestinal endoscopy practice. In the present assessment the sensitivity of eUAB, Pronto Dry and histopathology was low compared to 16S PCR.[14] This might have been due to a low viable bacterial cell count, sampling error, or inadequate size of biopsy specimen. Also, the use of PPI before endoscopy is known to reduce the sensitivity of the urease test to detect *H. pylori*;[15–17] however, patients with prior PPI use were excluded from the present study.

Test reading times for eUAB of 30 and 60 min (as recommended for Pronto Dry) gave optimal sensitivity and specificity, and provided adequate time for patients to recover from sedation. A single eUAB costs approximately US$4, compared to the US$20 for Pronto Dry. Thus, eUAB might be suitable when rapid diagnosis of *H. pylori* is required and there is also a need to keep the cost to a minimum.

Presently, histological examination of gastric biopsy is considered to be the most accurate method for *H. pylori* detection. However, expertise and experience are required and this adds US$20 to the overall cost of the examination (US$85). Non-invasive procedures such as the *H. pylori* stool antigen (HpSA) test and the urea breath test (UBT) have been introduced recently in Pakistan and are undergoing controlled trials.[18,19]

In conclusion, this study shows that eUAB can be used as a rapid urease test for *H. pylori* and has a sensitivity and specificity comparable with commercially available alternatives. However, a larger study is required to confirm these results. □

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