Helicobacter pylori in Dyspepsia: Phenotypic and Genotypic Methods of Diagnosis

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Abstract

Background: *Helicobacter pylori* affects almost half of the world's population and therefore is one of the most frequent and persistent bacterial infections worldwide. *H. pylori* is associated with chronic gastritis, ulcer disease (gastric and duodenal), mucosa-associated lymphoid tissue lymphoma, and gastric cancer. Several diagnostic methods exist to detect infection and the option of one method or another depends on various genes, such as availability, advantages and disadvantages of each method, monetary value, and the age of patients. **Materials and Methods:** Patients with complaints of abdominal pain, discomfort, acidity, and loss of appetite were chosen for endoscopy, detailed history was contained, and a physical examination was conducted before endoscopy. Biopsies (antrum + body) were received from each patient and subjected to rapid urease test (RUT), histopathological examination (HPE), polymerase chain reaction (PCR), and culture. **Results:** Of the total 223 biopsy specimens obtained from dyspeptic patients, 122 (54.7%) were positive for *H. pylori* for HPE, 109 (48.9%) by RUT, 65 (29.1%) by culture, and 117 (52.5%) by PCR. The specificity and sensitivity were as follows: RUT (99% and 88.5%), phosphoglucosamine mutase PCR assay (100% and 95.9%), and culture (100% and 53.3%), respectively. **Conclusion:** In this study, we compared the various diagnostic methods used to identify *H. pylori* infection indicating that, in comparison with histology as gold standard for detection of *H. pylori* infection, culture and PCR showed 100% specificity whereas RUT and PCR showed 99% and 100% sensitivity, respectively.

Keywords: Biopsy, culture, diagnosis, *Helicobacter pylori,* histopathology, phosphoglucosamine mutase gene, rapid urease test

INTRODUCTION

Helicobacter pylori is a microaerophilic spiral-shaped Gram-negative bacterium which colonizes the human gastric mucosa. It is an etiological agent of chronic active gastritis, gastric and duodenal ulcers, mucosa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma.[1-4] The prevalence of *H. pylori* infection is declining in developed countries (25%–50%) but increasing in developing countries (70%–90%), because of the overcrowded conditions and poor socioeconomic status.[5] The most likely mode of transmission is person-to-person spread, but oral-oral and fecal-oral transmissions have also been described.[6,7]

Diagnostic approaches to identify *H. pylori* infection are varied, and the selection of one method or another depends on numerous factors, such as the accessibility of diagnostic tests, need to perform an endoscopy, advantages, disadvantages, and cost of each method, and also patient's age. At present, there are both invasive and noninvasive techniques to diagnose

H. pylori infection. Invasive methods such as histology, rapid urease test (RUT), microbiological culture, and polymerase chain reaction (PCR) require endoscopy and are well known as biopsy-based examination. Noninvasive tests include stool antigen test, serology, and urea breath test (UBT). Some elements which determine the selection of a given test strategy include sensitivity, specificity, clinical circumstances, and cost-effectiveness of the trial.[8,9] Notably, all these techniques have their own restrictions.

In countries like India, where endoscopy is routinely done, one of the most commonly used techniques is histopathological diagnosis. An experienced pathologist and quality of biopsies are two basic prerequisites for the proper histopathological

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examination (HPE). Improper biopsies, observer-related factors, topographical changes in the stomach, *H. pylori* density and its patchy distribution, and type of stain used may cause false results.[7,8] Culturing from gastric tissue is regarded as a definite proof of *H. pylori* infection. Since this method is more technically demanding, the ability to culture and the sensitivity of the examination may vary between laboratories. In clinical practice, the most routinely used technique is RUT. However to detect *H. pylori* from RUT, there should be sufficient bacterial load consisting of at least 104 bacteria.[10] Thus, the examination is less advisable for post-eradication follow-up since this measure may not be present after about 4 weeks of the failure of eradication therapy.

Many PCR methods have been evolved to find the organism directly from clinical specimens.[11,12] The *ureC* gene has been demonstrated to encode the phosphoglucosamine mutase (glmM), which is unrelated to urease production, and was renamed the *glmM* gene. *glmM*, housekeeping gene, is more appropriate method compared to other PCR methods for detection of *H. pylori* from clinical specimens.[11]

The purpose of the present study is to disclose the relationship between different *H. pylori* infection diagnosis (invasive) methods (histology, RUT, culture, and PCR) from direct biopsy specimens of dyspeptic patients and also to compare the sensitivity and the specificities of different methods for detection of *H. pylori* in gastric biopsy specimens and to clarify the application range of each diagnosis method and its determining factors in India.

MATERIALS AND METHODS

Specimen collection and processing
A total of 223 adult patients of both genders having complaints of abdominal pain, discomfort, acidity, and loss of appetite were chosen for endoscopy at Kasturba Hospital, Manipal, and detailed history was contained and a physical examination was carried out before endoscopy. Subjects who had received H2-receptor blockers, antimicrobial therapy, proton-pump inhibitors, and nonsteroidal anti-inflammatory drugs 30 days before endoscopy were excluded from the study. Patient's consent to take part in the study was obtained as per the protocol of the institutional ethical committee. Biopsies from the stomach (antrum + body) were obtained from each patient and one was subjected to RUT, the other one in 10% formalin and processed for HPE. One more in 120 µl phosphate-buffered saline (PBS) for PCR and an another one in 0.6 ml Brucella broth with 15% glycerol as transport media stored at −80°C until cultured.

Rapid urease test
The RUT was performed with a broth containing 0.1% (peptone, glucose), 0.5% NaCl, 0.2% KH_2PO_4, 2% urea, and 0.0012% phenol red, prepared freshly before endoscopy. One bit antral and body sample was placed in the solution and maintained at room temperature. The test was considered positive when the color changed from yellow to red within 24 h.

Histopathological examination
Two bits of tissue biopsies from both antrum and the body treated with formalin was stained routinely with hematoxylin and eosin, and special stains such as Giemsa were used as and when needed.

Helicobacter pylori culture
Transport medium containing the two bits of biopsy samples from both body and antrum was vortexed, and 100 µl of it was plated on brain–heart infusion agar supplemented with 0.4% IsovitaleX, 7% horse serum, and *H. pylori* dent supplement. Cultured plates were then incubated in a microaerobic atmosphere (5% O_2, 10% CO_2, and 85% N_2) at 37°C for 3–7 days. *H. pylori* was identified based on their distinctive morphology, Gram staining, and positive reaction for urease, oxidase, and catalase tests.

Extraction of genomic DNA
Biopsy specimens collected in microcentrifuge tubes containing 120 µl of sterile PBS were vortexed vigorously for 2 min. The centrifuge tubes were then boiled in a water bath for 15 min, cooled in ice, and centrifuged at $13,000 \times g$ for 1 min. The supernatant (genomic DNA) was transferred to another tube.[12]

Polymerase chain reaction amplification
Amplification of *glmM* gene of *H. pylori* by PCR assay was as follows: final volume of 25 µl reaction mixture contained 3 µl template DNA, 10 µl PCR Master Mix (sigma), 1 µl forward (5' AAGCTTTTAGGGGTGTTAGGGGTTT 3') and (5' AAGCTTACTTTCTAACACTAACGC 3') reverse primers[13] and 5 µl molecular grade water. The cycling conditions were as follows: initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, with final extension at 72°C for 7 min.

Statistical analysis
Comparisons of validity of diagnostic test, specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) were done using histology as gold standard from IBM SPSS Statistics version 20.0.

RESULTS

A total of 223 dyspeptic patients were included in this study, of which 118 were positive for *H. pylori* by histopathology. In this study group, 82 male and 36 female patients ranging in age from 19 to 80 years were included in the study. Endoscopy was done; (antrum and body) biopsies were collected from 223 patients. Identification of *H. pylori* was performed on all gastric biopsies by RUT, histopathology, PCR, and culture.

RUT was observed within a few minutes up to 24 h; the rate of positive *H. pylori* in the biopsies tested was 109 (48.9%). HPE results as positive and negative were received after few days of endoscopy, and positive rate was 122 (54.7%). Housekeeping gene, *glmM*, was detected by PCR on agarose

gel as a band with 294 bp in size [Figure 1] and positivity was 117 (52.5%). The culture of the biopsy samples yielded a positive of 65 (29.1%) after incubation for a period of 3–7 days. The overall rate of detection of *H. pylori* from biopsy specimens of dyspeptic patients is shown in Table 1.

Histological examination results were evaluated as the gold standard, and specificity, sensitivity, PPV, and NPV were calculated for each test [Table 2].

DISCUSSION

In the clinical scenario, a quick and economical finding method for analysis of *H. pylori* infection is anticipated. *H. pylori* infection can be detected by a variety of methods.[10] In the tedious medical diagnostics, the urease test, histological examination, PCR, bacterial culture, UBT, stool antigen detection, and serology are valuable methods of detecting *H. pylori* infection. In the present study, we have adapted the invasive (biopsy) methods of detecting *H. pylori* and have concluded that histopathology and PCR are the better diagnostic methods than RUT and culture.

Histopathology has generally been seen as being the first diagnostic method for *H. pylori* detection and is still widely used means of diagnosis.[14] With this tool, the histopathologist can recognize the distinctive morphology of *H. pylori* and at the same time evaluate the pattern of gastritis and/or recognize coincidental premalignant (dysplastic) changes or frankly neoplastic lesions. The sensitivity of the histology is generally 90–95%, and the specificity is 95–98%; it will in turn vary with biopsy and observer-related factors. It is also possible to have sampling errors due to differences in colonization density. However, several previous studies display important interobserver variation, suggesting that the accomplishments of the pathologist are impressive when it comes to the histopathological diagnosis of *H. pylori*.[10,15,16] Therefore, in the present study, we have seen that histology is an ideal method of detecting *H. pylori*.

The specificity of RUTs ranges from 95% to 100% while the sensitivity is 80%–95%.[17] The sensitivity is affected primarily by the number of bacteria present in the biopsies. Chances of false-negative result in urease may be obtained in patients with achlorhydria as well as in patients on proton-pump inhibitors because of the increased luminal

pH that can lead to extremely high pH adjacent to the organism, such that *H. pylori* is destroyed by the natural process of its own urease. In the present study, the sensitivity of RUT was 88.5%, which is very snug to those by other authors.[10,18,19] Likewise, the specificity of RUT is rather the same as those described by other workers, though we did not observe any false positive result by RUT in contrast with the other studies.

H. pylori from the culture is definitive evidence of infection. Nevertheless, the skill to isolate the organism from infected subjects differs broadly between laboratories and makes it the most technically challenging diagnostic test. The viability of the bacterium will be lost mainly due to the exposure to the surrounding environment, unable to transport quickly to laboratory for culture. Numerous transport media have been specified, such as normal saline, Brucella broth, Stuart's transport medium, chocolate agar slants, and 20% glucose. *H. pylori* can be stored for longer period by making glycerol broth stored at −80°C. Selectivity can be obtained by adding different combinations of antibiotics to culture media.[10,20] Therefore, culture is 100% specific in diagnosing *H. pylori* infection. Unfortunately, between centers due to local of expertise, sensitivity can alter widely. Nevertheless, these techniques demand money, time, and personnel, and their purpose is restricted primarily to research centers.

Molecular methods have the advantage of their quickness and the limited influence of the transport conditions. To date, many PCR methods have been introduced to detect the organism directly from the clinical samples. Depending on the primer used, the method has a reported sensitivity of 10–100 bacteria. A variety of genes including the 16S rRNA, random sequence, *ureC* (*glmM*) gene, *ureA* gene, and the 26-kDa species-specific antigen gene have been used as targets. A study done elsewhere shown that the *ureC* amplifications have more sensitivity compared to others and obtained only with *H. pylori*, while none of the other urease-positive or related bacteria that had been tested gave the expected amplified DNA products.[11,12] Our study reconfirms the sensitivity and the specificity of the PCR assay with the *ureC* (*glmM*) primers.[9,11,21]

CONCLUSION

However, to date, the organization might vary slightly among similar works on *H. pylori* diagnosis. Only the general level is that in almost all studies, biopsy-based methods are chosen over other methods; none of these methods can be used as the gold standard entirely. Therefore, concurrent utilization of biopsy-based and noninvasive methods is recommended for *H. pylori* infection confirmation. To conclude by the experience of present study, we recommend Histopathology and PCR as the most reliable tests for diagnosing *H. pylori* from biopsy specimens.
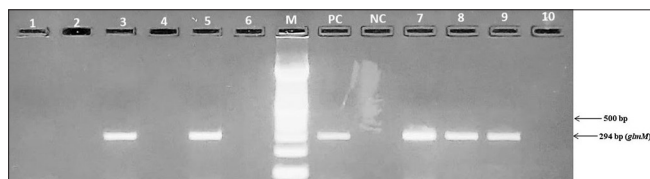

Figure 1: Polymerase chain reaction products for *Helicobacter pylori* with phosphoglucosamine mutase gene-based primers. Lane NC, negative control; Lane PC, positive control; Lane 3, 5, 7, 8, 9 positive patients biopsy sample; Lane 1, 2, 4, 6, 10 negative patients biopsy samples; Lane M, ladder

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Table 1: Results of four different methods for the detection of *Helicobacter pylori* in 223 gastric biopsy specimens

Number of specimens (biopsy)	RUT		Histopathology		Culture		PCR (*glmM* gene)	
	Positive, *n* (%)	Negative, *n* (%)	Positive, *n* (%)	Negative, *n* (%)	Positive, *n* (%)	Negative, *n* (%)	Positive, *n* (%)	Negative, *n* (%)
223	109 (48.9)	114 (51.1)	122 (54.7)	101 (45.3)	65 (29.1)	146 (70.9)	117 (52.5)	106 (47.5)

RUT: Rapid urease test, PCR: Polymerase chain reaction

Table 2: Sensitivity, specificity, positive and negative predictive values of the diagnostic methods statistical analysis according to standard test

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Culture	53.3	100	100	63.9
Biopsy PCR	95.9	100	100	95.3
RUT	88.5	99	99.1	87.7

PPV: Positive predictive value, NPV: Negative predictive value, PCR: Polymerase chain reaction, RUT: Rapid urease test

Conflicts of interest

There are no conflicts of interest.

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