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Comparison of five diagnostic methods for *Helicobacter pylori*

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ABSTRACT

Background and Objectives: Invasive and non-invasive techniques are used to diagnose *H. pylori* infection. Some factors influence the choice of a diagnostic test, such as the sensitivity and specificity of the tests, the clinical circumstances and the cost-effectiveness of the testing strategy. The aim of this study was to reveal the relationship between different *H. pylori* infection diagnosis methods, and clarify the application scope of each diagnosis method.

Materials and Methods: 91 patients were included in the study, and specimens including biopsies, blood and stool were taken. Biopsies were evaluated by hematoxylin and eosin, and Giemsa staining. A sequence of 294 bp in the *ureC* (*glmM*) gene was amplified. The rapid urease test (RUT) was performed using a non-commercial validated test. Stool samples were analyzed using a polyclonal ELISA stool antigen test. A serological assay for IgG antibodies was performed by a commercial *Helicobacter pylori* IgG ELISA kit.

Results: According to the predefined criteria, a total of 46 (50.5%) patients tested were positive by at least 2 of the 3 biopsy-based methods. The best sensitivity (95.6%) belonged to histology and RUT. The sensitivities of other tests including PCR, serology and stool antigen test were 93.5%, 91.3% and 73.9%, respectively. RUT showed the best specificity (100%), and the specificities of the other tests, including PCR, stool antigen test, histology and serology, were 95.6%, 86.7%, 77.8% and 55.6%, respectively.

Conclusion: In view of the better results obtained for invasive vs. non-invasive tests, for a more accurate diagnosis, it is advisable not to solely rely on non-invasive methods of *H. Pylori* diagnosis.

Keywords: *Helicobacter pylori*, Histopathology, Stool antigen test, ELISA, RUT, PCR

INTRODUCTIN

Helicobacter pylori is a microaerophilic spiral shaped gram-negative bacterium which colonizes the human gastric mucosa (1). It is regarded as the major cause of duodenal ulcers, gastric and gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma. The prevalence of *H. pylori* infection is 25%-50% in developed countries and

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70%-90% in developing countries (2-3). The most probable mode of transmission is person-to-person spread but oral-oral and fecal-oral transmissions have also been reported (4).

Invasive and non-invasive techniques are used 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 also known as biopsy-based tests. Non-invasive tests include stool antigen test, serology and urea breath test (UBT). Some factors which influence the choice of a given testing strategy include sensitivity, specificity, the clinical circumstances and the cost-effectiveness of the test (5). Notably, all these techniques have their own limitations (6). In countries where endoscopy is frequently performed, one of the most commonly used techniques is histopathological diagnosis. Experienced pathologist and quality of biopsies are two basic requirements for the proper histopathological examination. 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 (3).

Bacterial culturing from gastric biopsies is regarded as a definite proof of *H. pylori* infection. Since the method is more technically demanding, the ability to culture and the sensitivity of the test may vary between laboratories (7). In clinical practice, the most routinely used technique is RUT. However, to obtain a sufficient sensitivity, there should be sufficient bacterial load consisting of at least 10^5 bacteria. Therefore, the test is less advisable for post-eradication follow-up since this amount may not be present after about 4 weeks of the failure of eradication therapy (8-9).

In individuals not undergoing gastroscopy, serology is the easiest way to detect *H. pylori* infection by detecting circulating antibodies against *H. pylori*. However, it cannot differentiate between active and asymptomatic colonization and past and current *H. pylori* infection (10).

UBT has greater sensitivity and specificity than other non-invasive tests but its specificity is decreased when other urease producing bacteria are present in the human gut (11). It also needs more expensive and complicated equipments.

The aim of the study was to reveal the relationship between different *H. pylori* infection diagnosis methods (Histology, RUT, PCR, stool antigen test and serology) and to clarify the application scope of each diagnosis method and its influencing factors.

MATERIALS AND METHODS

Patients and samples. Totally, 91 patients who presented for routine upper gastrointestinal endoscopy at Firoozgar Hospital (a University hospital) and were willing to cooperate in this study were included. The patients included 39 males and 52 females with a mean age of 45 years (range, 17-87 years). Subjects who had received antimicrobial therapy, H2-receptor blockers, proton-pump inhibitors and non-steroidal anti-inflammatory drugs 30 days prior to endoscopy were excluded from the study. The ethics committee of the university granted approval for the study and all the patients gave their consent to participate.

Several biopsy specimens were obtained: one was reserved for RUT, and some, derived from the antrum and corpus, for histology (formalin-fixed and paraffin-embedded) and finally one for PCR. Stool specimens and serum samples from these patients were collected and kept on -20°C until used.

Rapid urease test. The RUT was performed using a non-commercial validated test. This test was performed with a homemade solution with 1 ml distilled water, one drop of 1% phenol red, and 100 mg urea, prepared just before endoscopy. One antral 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 hours (12).

Histopathology. Biopsies from the antrum and the corpus were obtained for histology and were fixed in 10% formalin and sent to the laboratory. Paraffin embedded and multiple 4 mm-thick histological sections were obtained from each biopsy. Preparations were stained with hematoxylin and eosin, and Giemsa evaluated by several pathologists blinded to the results of the other tests. The presence of *H. pylori* was determined but not graded.

PCR. DNA was extracted from biopsies using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). A sequence of 294 bp in the *ureC* (*glmM*) gene was amplified as described previously (13). Primer pair used for *ureC* amplification had the nucleotide sequence as follows: forward primer,(5′-AAGCTTTTAGGGGTGTTAGGGGTTT-3′) and reverse primer (5′-AAGCTTACTTTCTAACACTAACGC-3′). The

Table 1. Comparison of five different methods for diagnosis of *H. pylori* infections by gold standard.

Methods		Gold standard		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
		Positive	Negative					
	Positive	44	10					
Histology				95.6	77.8	81.5	94.6	86.8
	Negative	2	35					
	Positive	44	0					
RUT				95.6	100	100	95.7	97.8
	Negative	2	45					
	Positive	43	2					
PCR				93.5	95.6	95.6	93.5	94.5
	Negative	3	43					
	Positive	42	20					
Serology				91.3	55.6	67.7	86.2	73.6
	Negative	4	25					
	Positive	34	6					
Stool antigen test				73.9	86.7	85	76.5	80.2
	Negative	12	39					
Total		46	45					

PCR conditions consisted of 1 cycle of 5 min at 93°C, followed by 35 cycles of 1 min at 93°C, 30 s at 55°C, 30 s at 72°C, and a final cycle of 10 min at 72°C. Amplified products were visualized on 2% agarose gel under UV light. All assays were performed at least twice.

Stool antigen test. Stool samples were analyzed using a polyclonal ELISA stool antigen test (Astra s.r.l, Milan, Italy), according to manufacturer's instructions. Briefly, diluted fecal samples and peroxidase-conjugated polyclonal antibodies were added to the wells. After 90 minutes of incubation at room temperature, sample wells were washed to remove unbound samples and enzyme-labeled antibodies. The results were read at 450/620nm by spectrophotometry. To determine *H. pylori* antigen concentrations in test samples, a cutoff value of OD 0.2 was used. Samples with OD values < 0.150 were considered negative. Samples with OD values within 0.150-0250 were considered borderline and samples with OD values > 0.250 were considered positive (14).

Serology. On the endoscopy day, 5 ml blood was taken from patients and transferred to the laboratory. The sera were separated and kept until the day of testing at -20°C. A serological assay for IgG antibodies against *H. pylori* was performed by a commercial *Helicobacter pylori* IgG ELISA kit (IBL, Hamburg, Germany) according to the manufacturer's instructions. The results were classed as positive if anti–*H pylori* immunoglobulin (Ig) G titers were >12 U/ml, negative if they were < 8 U/ml, and equivocal if they were between 8 and 12 U/ml.

RESULTS

The positive and negative histology results were received within the next few days. RUT results were observed within a few minutes up to 24 hours. Positive PCR results were observed on agarose gel as a band at with 294 bp in size (Fig. 1). As the culture of *H. pylori* from biopsies was not performed in our study, other endoscopic-based techniques (RUT, PCR and histological staining of the biopsies) were considered the gold standard for determination of the specificity and sensitivity of each test. Patients were considered to be infected with *H. pylori* if 2 of 3 tests were positive. Based on the stated criteria, 46 (50.5%) of the patients were diagnosed as *H. pylori* infected and 45 (49.5%) as uninfected.

Sensitivities, specificities, predictive values and accuracy of the biopsy-based and the ELISA-based diagnostic assays calculated for all 91 patients in relation to the gold standard are presented in Table 1.

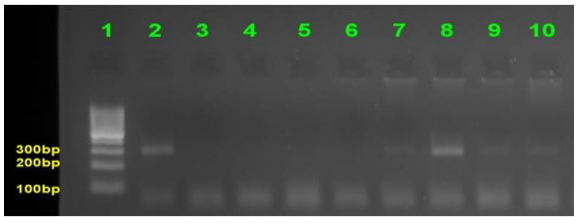

Fig. 1. PCR products for *H. pylori* with *glmM* gene based primers. Lane 1, ladder; Lane 2, positive control; Lane 3, negative control; Lane 4-10, Patients biopsy samples.

DISCUSSION

In the clinical setting, a rapid and cost-effective detection method for diagnosis of *H. pylori* infection is desirable. *H. pylori* infection can be detected by a variety of methods (15). In the routine clinical diagnostics the urease test, histological examination, urea breath test, serology, bacterial culture and stool antigen test are valuable methods of detecting *H. pylori* infection.

Histopathology has historically been considered as being the first diagnostic method for *H. pylori* detection and is still widely used as the main diagnostic tool in suspicious patients with upper gastrointestinal symptoms or in highly prevalent areas (16). Correct and trustworthy histologically diagnosing of *H. pylori* gastritis has a high influence on clinical practice as a therapeutic indicator. Nevertheless, several previous studies display important inter-observer variation, suggesting that the skills of the pathologist are impressive when it comes to the histopathological diagnosis of *H. pylori* (17-18). In the present study, one pathologist would normally found more positive results when the other tests were negative, suggesting that the experience and skills of pathologist do matter for the specificity and sensitivity of histopathological examination (19).

In the present study, the sensitivity of rapid urease test was 95.6%, which is very close to those by other authors (14, 20-22). Also, the specificity of RUT is rather the same as those reported by other workers, though we did not get any false positive result by RUT in contrast with the other studies (14, 20, 22).

Molecular methods have the advantage of their rapidity and the limited influence of the transport conditions. To date, many PCR methods have been developed to detect directly the organism in clinical samples. A variety of genes including the *cagA* gene, the *ureC* (*glmM*) gene, the *ureA* gene, the 16S rRNA gene and the 26-kDa species-specific antigen (SSA) gene have been used as targets. Lage and colleagues showed that the *ureC* amplifications were 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 (23). Our study re-confirms the sensitivity and the specificity of the PCR assay with the *ureC* (*glmM*) primers (6, 13, 23).

Many serological tests are available commercially. They are widely in use because they are inexpensive and easy to use. However, since antibody titers can remain high for months after elimination of infection (24), the sort of the tests based on the detection of specific antibodies are not reliable to check eradication of *H. pylori* (25). In this study, serology showed the lowest specificity and accuracy in comparison with other tests. Generally, low accuracy of IgG serological tests is due to the inability to differentiate between current and past infection. In contrast, considering that almost all previously treated participants were excluded by the questionnaire, and those *H. Pylori* infection was rarely cured spontaneously, 11 of the single positive serology test might reflect past infection and/or false positive test result (14).

To date, several stool antigen tests have been developed commercially. Despite the heterogeneity in reported sensitivity and specificity rates, most of them have acceptable results and many studies have claimed that the stool antigen test is useful for the primary diagnosis and post-treatment follow-up of *H. pylori* infection (26). Premier Platinum HpSA as the first and the most used valid *H. pylori* stool antigen test, has been recommended as a reliable alternative to UBT in the initial diagnosis and follow-up period (27-28); but there are a number of studies reporting a lower level of accuracy (29-30). In our study, stool antigen test showed the lowest sensitivity (73.9%), and not so good specificity (86.7%). Upon our search, we could only found one study which had used this sort of kit. Despite nearly identical conditions, their results were better than that found in our study (85% for sensitivity and 90% for specificity)(31). The accuracy of the test might change from lot to lot and intertest variability has already been reported by Makristhatiset al.(32). Therefore, such discrepancies could be assigned not only to methodological failures but also to intertest variability(33).

Based on the results provided by this study, the accuracy of the tests for *H. pylori* diagnosis can be arranged in order as follows: RUT>PCR>histology>stool antigen test>serology.

However, the arrangement might change slightly among similar studies. But the general point is that in almost all studies, biopsy-based methods are preferred over other methods; and none of these methods can be considered as the gold standard alone. Thus, simultaneous utilization of biopsy-based and non-invasive methods is recommended for *H. pylori* infection confirmation.

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