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BRIEF ARTICLE

Five methods for detection of *Helicobacter pylori* in the Turkish population

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Abstract

AIM: To compare culture analysis, *Helicobacter pylori* (*H. pylori*) stool antigen (HpSA) test, polymerase chain reaction (PCR) and fluorescence *in situ* hybridization (FISH) for *H. pylori* detection.

METHODS: One hundred and thirty-two consecutive adult dyspeptic patients receiving diagnostic endoscopy at the department of gastroenterology were enrolled in this study. Culture and histological examination were performed on biopsy specimens. PCR and FISH tests were applied to histopathological samples. Stool samples that were simultaneously collected were tested for the *H. pylori* antigen using the HpSA test and bacterial DNA using stool PCR.

RESULTS: *H. pylori* was positively identified by histological examination in 85/132 (64.4%) of the patients, while positive samples were found in 56 (42.4%), 64 (48.5%), 98 (74.2%), 28 (21.2%) and 81 (61.4%) of the patients by culture, HpSA, PCR, stool PCR and FISH methods, respectively. The results of the culture, biopsy PCR, HpSA and FISH tests, with the exception of the stool PCR, were found to correlate with the histological examination as a gold standard.

CONCLUSION: The HpSA test is a rapid, simple, and noninvasive test for monitoring therapy. FISH is an accurate, rapid, cost-effective, and easy-to-use test for *H. pylori* detection.

Key words: *Helicobacter pylori*; Histology; Polymerase chain reaction; *Helicobacter pylori* stool antigen; Fluorescence *in situ* hybridization

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INTRODUCTION

In 1984, Marshall and Warren[1] reported the discovery of a bacterium, which was subsequently named *Helicobacter pylori* (*H. pylori*)[2], whose habitat was the human gastric mucosa. This bacterium has been shown to play a role in gastritis, peptic ulcer disease, and gastric malignancies[3-5]. Colonization of the human gastric mucosa induces chronic gastritis and peptic ulcer disease[3,4]. In addition,

H. pylori plays a role in the etiology of gastric cancer and cancer of the mucosa-associated lymphoid tissue[5].

The accurate detection of *H. pylori* is essential for the management of patients and for the eradication of the bacterium following treatment. Since the discovery of *H. pylori*, several diagnostic methods have become available for determining the presence of *H. pylori* infection. These tests can be assessed by invasive and noninvasive methods[6]. Assessment of *H. pylori* infection is based on noninvasive tests, such as serological methods, C urea breath test, and bacterial DNA sequences or bacterial antigen detection in stool by the *H. pylori* stool antigen (HpSA) test[7]. Under many circumstances, noninvasive testing is preferred. These tests are attractive because of their simplicity and the ability to provide test results within a few minutes after administration, in a physician's office. In contrast, the direct detection and culturing of *H. pylori* for the diagnosis of infection requires gastric biopsy specimens obtained from invasive gastroduodenoscopy[5]. Culture methods require an incubation period of at least 4-7 d. However, it is important to note that *H. pylori* is a fastidious microorganism and is affected by environmental conditions[8,9]. The presence of *H. pylori* or resistance to antimicrobials can be investigated on gastric tissue samples with molecular methods, such as polymerase chain reaction (PCR) and fluorescence *in situ* hybridization (FISH).

The aim of this study was to compare culture analysis, HpSA test, PCR and FISH to histological examination for the detection of *H. pylori*.

MATERIALS AND METHODS

Clinical samples

One hundred and thirty-two consecutive adult dyspeptic patients receiving diagnostic endoscopy at the department of gastroenterology were enrolled in this study. Written informed consent was obtained from all of the patients before endoscopy, and sample collection and approval by the Local Ethical Committee was taken prior to initiation of the project. Patients who underwent partial or complete gastrectomy, those with prior *H. pylori* eradication therapy, or those who were treated with any antibiotics, colloidal bismuth compounds, proton pump inhibitors, or H_2 receptor blockers within the past 4 wk were excluded from the study.

Endoscopy and biopsy sampling

Endoscopy was performed with a PentaxFG-29W (Pentax, Germany) on patients after an overnight fast. Four gastric biopsies (two from the antrum and two from the corpus) were taken from each patient.

Culture

Two gastric biopsy specimens, one from the antrum and one from the corpus, were obtained and placed in Stuart's transport medium. Cooled samples were transported to the laboratory of the Department of Microbiology within 1-2 h after procurement, as previously described[10]. Specimens were inoculated onto brain-heart infusion agar supplemented with sheep blood (10%), vancomycin (10 mg/L), trimethoprim lactate (5 mg/L), cefsoludin (5 mg/L), and amphotericin (5 mg/L). The plates were microaerobically incubated using CampyGen (Oxoid, United Kingdom) at 37 ℃ for up to 7 d. Positive cultures were identified by colony formation and Gram stain morphology as well as positive catalase, oxidase, and urease tests.

Histology

Two gastric biopsy specimens, one from the antrum and one from the corpus, were fixed in 10% formalin in separate containers and were sent to the Pathology Laboratory. Samples were embedded in paraffin wax, cut at 5 μm thickness, and stained with modified giemsa and hematoxylin and eosin. Histological evaluation of the samples for *H. pylori* was performed according to the Modified Sydney system[11]. The pathologist was unaware of the patients' clinical conditions and other test results.

HpSA

Stool samples were tested for *H. pylori* antigen by the monoclonal antigen FemtoLab *H. pylori* Cnx kits (Connex GmbH, Martinsried, Germany) using the manufacturer's protocol. Approximately 0.1 g of stool sample was added to vials that contained 1 mL of sample diluent and then emulsified by vortexing for 15 s. The tip of the vial was snapped off and 50 μL sample and 50 μL conjugate were added to the test well. The strip was rinsed after incubation for exactly (60 ± 5) min at ambient temperature. After washing, 100 μL substrate was added and then incubated for 10 min. Finally, the stop solution was added and the samples were analyzed on a spectrophotometer at a wavelength of 450 nm.

PCR

Gastric biopsies from all of the study subjects were stored at temperatures at or below -70 ℃ until use. Each biopsy was digested with tissue extraction buffer at 55 ℃ for 3 h. Then, 200 μL phenol was added to the tissue lysate to extract genomic DNA. *H. pylori* genomic DNA from stool samples was extracted according to Gramley *et al*[12]. Genomic DNA was subsequently quantified by PCR with 16S rRNA. Amplified fragments were separated on a 1% agarose gel and visualized under ultraviolet light.

Fluorescence in situ hybridization

Formalin-fixed paraffin-embedded gastric biopsies were sectioned and dehydrated. The sections were then air-dried and hybridized using the commercially available test system Seafast® *H. pylori* Combi Kit (Izinta, Hungary) according to the manufacturer's instructions. The oligonucleotide probe Hpy-1, which targets a specific sequence of 16S rRNA from *H. pylori*, was hybridized to the sections. Evaluation was performed with a fluorescent microscope equipped with a filter for green fluores-

Table 1 Statistical analysis according to standard test

Method	Sensitivity	Specificity	PPV	NPV	OR	RR
Culture	0.6118	0.9149	0.9286	0.4342	16.94	2.14
HpSA	0.7222	0.6667	0.8125	0.4545	5.20	1.79
Biopsy PCR	0.8824	0.5106	0.7653	0.2941	7.83	2.60
Stool PCR	0.2118	0.7872	0.6429	0.6442	0.99	1.00
FISH	0.9294	0.9574	0.9753	0.1176	296.25	8.29

PPV: **Positive predictive value; NPV: Negative predictive value; OR:** Odds ratio; **RR: Relative risk; HpSA:** *Helicobacter pylori* stool antigen; **PCR:** Polymerase chain reaction; **FISH: Fluorescence** *in situ* hybridization.

Table 2 Test results compared to standard test

Method	False positive	False negative	*r*	*P* value
Culture	4	33	0.510[1]	< 0.001
HpSA	12	20	0.276[1]	< 0.002
Biopsy PCR	23	10	0.430[1]	< 0.001
Stool PCR	10	67	0.001	> 0.05
FISH	2	6	0.872[1]	< 0.001

[1]Correlation is significant at the 0.01 level. HpSA: *Helicobacter pylori* stool antigen; **PCR: Polymerase chain reaction; FISH: Fluorescence** *in situ* hybridization.

cence (Nikon Eclipse 600, Japan).

Statistical analysis

The χ^2 and Pearson correlation analysis were conducted and the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), *P* value, *r* value, odds ratio (OR) and relative risk (RR) were calculated using standard formulas for data using SPSS v. 10.0 (IBM, United States).

RESULTS

H. pylori was identified by histological examination in 85/132 (64.4%) of the patients, while 47/132 (35.6%) of the patients were classified as *H. pylori* negative. Furthermore, positive results were obtained in 56 (42.4%), 64 (48.5%), 98 (74.2%), 28 (21.2%) and 81 (61.4%) of patients by the culture method, HpSA analysis, PCR, stool PCR and FISH, respectively. Histological examination results were evaluated by the gold standard, and specificity, sensitivity, PPV and NPV were calculated for each test (Table 1). A high number of false-positive results was observed in the biopsy PCR (23/98; 23.4%). However, a higher rate of false-negative results was obtained with the culture method (33/76; 43.4%). The culture method, biopsy PCR, HpSA and FISH tests were found to correlate with the Pearson correlation analysis. Similarly, these tests were statistically comparable to the histological examination based on the *P* value with the χ^2 test. In contrast, the stool PCR test did not correlate or have a significant *P* value. These data are summarized in Table 2.

DISCUSSION

There are currently several different diagnostic tests that exist for detecting *H. pylori* infection. Each test has its own merits and demerits in terms of indication, sensitivity, specificity, cost and time. Several studies have examined the diagnostic performance of invasive and non-invasive methods[6,7,9,12,13]. However, these studies were biased or demonstrated a lack of agreement[13]. One possible reason for the discrepancies in diagnostic performance might be due to the selection of various reference methods. Currently, there is no established method to provide a definitive or standard diagnosis of *H. pylori* infection. The selection of tests or the use of a combination of tests without identifying any one specific test as a reference standard can introduce bias[14].

One limit of histological detection of *H. pylori* in gastric biopsy specimens is interobserver variability in assessment[15,16]. A meta-analysis has reported that histological examination results have an approximate sensitivity of 0.70 and specificity of 0.90[17]. This may be due to the discrepancies in the evaluation of features of *H. pylori* or the observations of the pathologist, because pathology results are based on subjective interpretation of different features and classification. Various studies on the reproducibility of histopathological data have reached a similar conclusion. However, the histological examination of the gastric biopsy specimen is accepted as the gold standard for the diagnosis of *H. pylori*[18]. In this study, histological examination resulted in 64.4% positivity for *H. pylori*, which showed a good correlation with the positive detection rates of other methods, with the exception of stool PCR.

Culturing biopsy specimens cannot be routinely used because it is time consuming and very difficult to maintain strict anaerobic conditions. However, bacterial cultures can surely provide specific results and informative data. Gisbert and Abraria have reported three studies with culture sensitivity of 0.45 and specificity of 0.98 in 2006[17]. Similarly, we found that the culture sensitivity and specificity was 0.61 and 0.91, respectively. In addition, the statistical analysis showed a PPV of 0.93, NPV of 0.43, OR of 16.94, and RR of 2.14 compared to histological examination.

The HpSA test is available and recommended in the Maastricht 2-2000 Consensus Report[19] for the pretreatment diagnosis of *H. pylori* infection and confirmation of a *H. pylori* cure following treatment. In a Japanese study, the HpSA test had a reported sensitivity of 93.9% and specificity of 95.7%, compared to a diagnosis of infection based on histological examination[20]. However, Blanco *et al*[21] have observed that another stool antigen test showed a low sensitivity (75%-79%), in patients with *H. pylori* infection who were tested after erradication therapy. We studied the accuracy of the HpSA test in the Turkish population. The HpSA test had a sensitivity of 0.72, specificity of 0.67, accuracy of 0.77, PPV of 0.81, OR of 5.20 and RR of 1.79. Thus, the HpSA test results had a low but acceptable correlation with the histological examination.

It has been reported that the FISH method is an accurate, inexpensive, rapid test for the detection of *H.*

pylori in paraffin-embedded gastric biopsy samples, with a high sensitivity and specificity[22-24]. In addition, it can be applied to fresh gastric tissue samples and *H. pylori* isolates from culture[25]. In this study, the FISH method had a strong correlation with the histological examination and exhibited a sensitivity of 0.93, specificity of 0.96, accuracy of 0.94, PPV of 0.98, OR of 296.25 and RR of 8.29. Furthermore, the FISH method may be a very useful *H. pylori* diagnostic tool in microbiology in the future.

In gastric tissue, the presence of *H. pylori* and resistance genes can be investigated by PCR. It has a high sensitivity and specificity, and can be used as a follow-up assessment after therapy[26,27]. In this study, biopsy PCR studies had a sensitivity of 0.88, specificity of 0.51, accuracy of 0.75, PPV of 0.77, OR of 7.83 and RR of 2.60. Moreover, we found that the specificity value was particularly low for the biopsy PCR results. However, there was a discrepancy between our study and previous reports in terms of the specificity of *H. pylori* detection[28,29]. Lunet *et al*[28] have reported a difference in *H. pylori* positivity by histology *vs* PCR from different populations, in Mozambique and Portugal of 63.7% *vs* 93.1% and 95.3% *vs* 98.1%, respectively. Two possibilities could explain this conflicting result. First, a low density of *H. pylori* colonization may explain the histological results. Alternatively, the PCR results may be reliable because of the use of a specific primer for the particular population.

The stool PCR results had a very low sensitivity and OR (0.21 and 0.99) and had no significant correlation with the histological examination. Previous studies and our data clearly indicate that there is no clinical value in the determination of *H. pylori* in human feces by PCR because of insufficient sensitivity, specificity, and accuracy[30].

There are a variety of tests available for the diagnosis of *H. pylori* infection. Therefore, it is important that laboratories choose the test or tests that are appropriate for the conditions of the laboratories, patient numbers, costs, and account for the need to prepare their own diagnostic algorithms.

In conclusion, the culture, biopsy PCR, HpSA test, and FISH methods for the detection of *H. pylori* in this study, with the exception of stool PCR, were found to correlate with histological examination as a gold standard. In addition, there was a conflicting result on biopsy PCR data when compared to histological examination. The HpSA test is a rapid, simple, and noninvasive test with acceptable results that can be used for monitoring therapy. The FISH method is an accurate, rapid, cost-effective and easy-to-use test for the detection of *H. pylori*, and also allows for the simultaneous determination of antibiotic resistance in the same gastric tissue. Therefore, histopathological examination as a gold standard and the FISH test may be the preferred methods to use together for the precise detection of *H. pylori*.

COMMENTS

Background

Helicobacter pylori (*H. pylori*) plays a role in gastritis, peptic ulcer disease and also gastric malignancies such as gastric cancer and cancer of the mucosa-associated lymphoid tissue. The accurate detection of *H. pylori* is essential for the management of patients and eradication of the bacteria following treatment.

Research frontiers

Since the discovery of *H. pylori*, several diagnostic methods have been become available for determining the presence of *H. pylori* infection. However, there is no established method to provide a definitive or standard diagnosis of *H. pylori* infection.

Innovations and breakthroughs

The fluorescence *in situ* hybridization (FISH) test is an accurate, rapid, inexpensive and easy-to-use method for the detection of *H. pylori*, and allows determination of antibiotic resistance in the same gastric tissue simultaneously. In this study, FISH correlated well with histological examination. Therefore, histological examination and the FISH test may be preferred together for the precise detection of *H. pylori*.

Applications

This study suggests that, laboratories choose the test or tests that are appropriate for their own conditions, patient numbers and costs, and have to prepare their own diagnostic algorithms.

Terminology

For the detection of *H. pylori*, culture, *H. pylori* stool antigen test, polymerase chain reaction and FISH were used with histological examination.

Peer review

This was an interesting study, although a few problems need to be resolved before publication. The most important point is the reliability of their gold standard. The reasons for the false-positive and false-negative results of each test should be discussed further.

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