ORIGINAL ARTICLE

Invasive Versus Noninvasive Methods for Diagnosis of *Helicobacter Pylori* infection

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ABSTRACT

Background: Helicobacter pylori (H. pylori) is one of the most widespread infectious organisms in the world that is related to many gastrointestinal diseases. There are numerous techniques available at the present time for identifying infections caused by H. pylori, each has advantages and disadvantages. **Objectives:** To compare invasive versus noninvasive techniques used for diagnosis of H. pylori infections. **Methodology:** The study was performed on 100 patients complaining of gastrointestinal problems undergoing GIT endoscopy admitted to Gastroenterology Department, Benha University Hospital. Two samples of gastric biopsy were taken from each patient ; one used for bacteriological culture and one used for Polymerase chain reaction (PCR), 100 stool samples were taken for stool antigen test (SAT), and 100 serum samples for Enzyme-Linked Immunosorbent Assay (ELISA). **Results:** Out of 100 biopsy samples, 71 (71%) had been positive for PCR, 70 (70 %) had been positive for culture. Out of 100 stool sample, 64 (64%) had been positive for SAT. Out of 100 serum samples, 68 (68%) had been positive for ELISA. In comparison with PCR, the culture showed 98.6 % sensitivity, 89.7 % specificity, and total accuracy of 96% then SAT with 88% accuracy and ELISA showed 86% accuracy. **Conclusion:** Considering PCR as the gold standard method for H. pylori diagnosis, the culture showed the highest overall performance followed by SAT and ELISA.

INTRODUCTION

Helicobacter pylori (*H. pylori)* is a human pathogen that its infection has been associated with number of gastroduodenal disorders as peptic ulcer, chronic active gastritis, atrophic gastritis and cancer stomach. In developed countries, the frequency of infection with *H. pylori* is 30% -50% while it is 70% - 90% in developing countries. The socioeconomic circumstances, degree of urbanization and sanitation and availability of sources of clean water are probably the causes for the wide variations in *H. pylori* prevalence [1].

Many gastroduodenal illnesses can be effectively managed with a perfect diagnosis of infections caused by *H. pylori*. There are a number of diagnostic procedures accessible to the identification of *H. pylori,* and each test has advantages and disadvantages in particular clinical contexts. Many techniques are developed to produce the more accurate outcomes, even though none of them can be regarded as the only accepted gold standard in the medical settings [2].

Invasive tests are done using specimens from endoscopic biopsy. They include rapid urease test (RUT), histology, culture and molecular techniques. In RUT, the bacterial load must be at least 10^5 bacteria. As a result, it is not advised to be utilized in the post-eradication follow-up because this bacterial level perhaps not identified until 30 days after the failure of therapy [3].

The microbiological culture from endoscopy based gastric biopsies, is regarded as a definite proof for *H. pylori* investigation. For the routine diagnosis, culture needs a particular transport medium, particular incubation conditions, and a specific growth medium. The benefit of isolating *H. pylori* in cultures is that it facilitates to perform antibiotic susceptibility testing, which aids clinicians in choosing the most appropriate antimicrobial drugs that help in treatment [4].

Real time polymerase chain reaction based on endoscopy is superior at detecting lower concentration of organism. In addition, PCR has important value in detecting virulence factors like CagA and VacA and also detecting point mutations that cause antibiotic resistance to the organism [5].

Serology, stool antigens test (SAT), and respiratory tests are noninvasive procedures.To diagnose *H. pylori,* the urea breath test (UBT) is considered as the gold standard noninvasive technique. However, when additional urease-producing microorganisms present in gastrointestinal tract, the specificity of the test is reduced [6].

In comparison, The SAT approach is the other main noninvasive technique. Two SAT subtypes are used, enzyme immunoassay (EIA) and immuno-chromatography assay (ICA). Upper gastrointestinal hemorrhage, Proton pump inhibitors (PPIs), antibiotics and bowel motions are some of the variables that affect SAT accuracy [7].

Many serological assays depending on the finding of anti-*H. pylori* antibodies are widely used for the diagnosis and EIA technique is the most popular and reliable method among them. Because they are quick, inexpensive, and patient-acceptable, serological tests are commonly employed in screening of the infection for epidemiological research. The immunogenic proteins VacA, UreA, CagA,, GroEL, and Omp have all being regarded as potential candidates to identify infections. [8].

Since *H. pylori* infection cannot yet be diagnosed using a single reliable test, the purpose of our study is to compare invasive versus noninvasive methods used to diagnose *H. pylori* infections.

METHODOLOGY

The current cross sectional study was done in the period between April 2023 and September 2023 on 100 patients (62 males and 38 females with a mean age of 35 years) complaining of gastric disorders and subjected to GIT endoscopy, admitted to Gastro-enterology Department, Benha University Hospital. A written informed consent was obtained from each participant before being included in our study after Benha University Research Ethics Committee authorized it under number (Rc. 1.4.2023).

Patients who had undergone endoscopy within 30 days of receiving antibiotic medication, non-steroidal anti-inflammatory drugs, proton-pump inhibitors or H2-receptor blockers were not included in our research. We also excluded cirrhotic patients, pregnant females, and critical neuropathic patients from the study.

Sampling:

a- Two Gastric biopsy samples (from antrum and corpus) from each patient obtained during upper GIT endoscopy by gastroenterologist. Sterilized tubes which contain the brain heart infusion (BHI) broth medium (ThermoFisher SCIENTIFIC, UK), and 5% of fetal bovine serum (biowest, USA) were used to collect biopsies and transported to laboratory.

b- One Biopsy sample stored at -80 ˚C until used for PCR, the second sample was kept at 4 ˚C up to 24 hours to do bacteriological culturing.

c- Stool samples were collected from the patients in a clean container and sent to laboratory to be stored at -20 ˚C until used.

d- Blood samples were collected from pateints under strict aseptic circumstances in scew capped plain tubes and sent to laboratory, serum separated and stored at -20 ˚C until used.

Bacteriological culture:

Biopsy samples were cultured on Columbia blood agar containing horse or sheep blood (ThermoFisher SCIENTIFIC, UK). The plates were incubated in a microaerobic atmosphere at 35 ºC to 37 ºC For 5-7 days. Growth of the organism had been confirmed by morphological characteristics and biochemical reactions such as positive oxidase, urease and catalase reactions [9].

Detection of *(CSTP), (Urea C)* and *(HP 16s)* genes using PCR:

- DNA extraction: It was done using ABT Bacterial DNA Mini Extraction Kit according manufacturer's instructions (Applied Biotechnology, Egypt). Purified DNA stored at -20 ºC till used.
- DNA amplification: was done in a thermal cycler (Biometra, Germany) using 2X TOPsimple™ DyeMIX-nTaq kit (enzynomics, Korea). The procedure followed the manufacturer's instructions and using primers for chemotaxis signal transduction protein (*CSTP*), urease C (*Urea C*) and 16S rRNA (*HP 16s*). (Table 1)
- Amplification was done by initial denaturation for 2 min at 95 °C, then 35 cycles including: (denaturation at 95 °C for 30 sec, an annealing temperature specific for each gene (according to table 1) for 1 min, an extension at 72 °C for 1 min /kb), then finally a final extension at 72 °C for 5 min. After being electrophoresed on 1.5% agarose gel, the amplified products were visualized using ethidium bromide (Fig 1).
- Samples were considered to be positive when at least two of three PCR findings were positive[10].

Table 1: Primers used in the study:

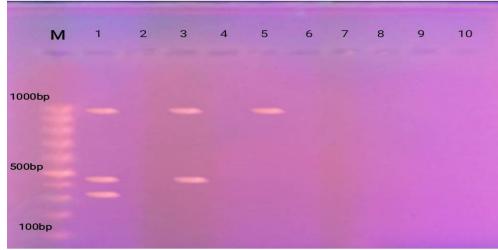| Target Genes	Sequences of primers (5–3)	Size of DNA amplicon (bp)	Annealing Temp (C°)	Reference
CSTP	Fw- GAAGTCATGGCTGATAGTTTA RvTAGTGCTGTATTTTTTCATGCTAA	987	59.8	11
HP16s	Fw-CAGCTTGTTGGTAAGGTAATGGC Rv-GATCTCTACGGATTTTACCCCTACAC	439	56	11
Urea C	Fw-CTAGTGGTGGTGGACAATTTAGG Rv-CTTGCTTACTTTCTAACACTAACGC	337	58	11

Fig. 1: PCR products of *(Urea C), (HP 16s)*, and *(CSTP)* genes
Lane M: 100 bp DNA ladder. Lane 1: amplified fragments of *Urea C* gene (337bp), *HP 16s* (439 bp) and *CSTP* gene (987bp). Lane 3: amplified fragments of *HP 16s* and *CSTP* genes. Lane 5: amplified fragment of *CSTP* gene.

Detection of *H. pylori* IgG by ELISA

Using the *H. pylori* IgG kit, ELISA was used to determine anti-*H. pylori* IgG presence. (Bioassay Technology Laboratory, UK) according to the directions of the manufacturer.

Stool Antigen test:

The test was preformed by immunochromatographic assay, using one step *H. pylori* Ag test (feces) (Sirin Diagnostics, Egypt).

Data analysis

The collected data was revised and tabulated using Statistical package for Social Science (IBM Corp. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). Data were presented and suitable analysis was done depending on the type of data obtained for each parameter.

RESULTS

The patients involved in the study were 62 males and 38 females with a spectrum of ages from 18-70 years.

Each test's results were displayed in (Table 2). Out of 100 gastric biopsy specimens, 71 (71%) were found positive by PCR and 29 (29%) were negative. The positive results for other tests were: 70 (70 %) for bacteriological culture, 64 (64%) for SAT and 68 (68%) for ELISA.

Table 2: The results of each *H. pylori* diagnostic test compared to PCR:

Technique	Positive	Negative	False positive	False negative	Total
PCR	71(71%)	29 (29%)	0 (0.0%)	0 (0.0%)	100(100%)
Culture	70 (70%)	26 (26%)	3 (3%)	1(1%)	
SAT	64 (64%)	24 (24%)	5(5%)	7(7%)	
ELISA	68 (68%)	18 (18%)	11(11%)	3(3%)	

Considering PCR as the gold standard method for diagnosis of *H. pylori*, The sensitivity, specificity, predictive values and accuracy of three different techniques which include culture, SAT and ELISA were determined to find the most appropriate test for the detection of *H. pylori* infection (Table 3). Compared to PCR, the bacteriological culture demonstrated the best overall performance with 98.6 % sensitivity, 89.7 % specificity, and accuracy of 96% followed by SAT with 88% accuracy and ELISA showed 86% accuracy.

Table 3: Test performance for each *H.pylori* diagnostic test compared to PCR:

Technique	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Test accuracy (%)	Disease prevalence (%)
Culture	98.6%	89.7%	95.9 %	96.3%	96%	71 %
SAT	90.1%	82.8%	92.8%	77.4%	88%	
ELISA	95.8%	62.1%	86.1%	85.7%	86%	

DISCUSSION

Although many techniques were established to identify *H. pylori*, the gold standard for diagnosing *H. pylori* infection is remain unclear. Each diagnostic technique has some limitations and is not totally appropriate in all circumstances. There isn't yet a single efficient method for determining *H. pylori* infection, despite the requirement for quick, highly accurate and cost effective tests in clinical settings [10].

Molecular methods have superior results than other tests due to speed of the test, no restrictions on sample transfer and a high level of accuracy. The PCR can directly detect microorganism that may present in the clinical samples [12].

Several genes can be used as targets for PCR. Since no primer exhibits 100% sensitivity or specificity, application of a single pair of primers is insufficient for identifing *H. pylori* infection. The *urea C*, *16S rRNA*, and chemotaxis signal transduction protein genes (*CSTP*) were used in the present study for detecting infection by various strains of *H. pylori.* A minimum two of them should be positive to consider the patient infected [10].

However PCR described as specific and sensitive test in multiple studies, a drawback of PCR is that patients must have oral endoscopy, as opposed to noninvasive assays like ELISA. The widespread endoscopy using is not only impracticable, but some people are unable to tolerate it. Accordingly, based on a "test-and-treat" strategy, patients could undergo noninvasive screening for *H. pylori* infection [2].

Since the presence of polymerase enzyme inhibitors, which have a negative impact on the test outcomes, the sensitivity of PCR may be diminished. Additionally PCR cannot distinguish between living and dead organisms and it may produce false-positive results [13].

Due to the bacterium's fastidious nature and specific growth needs of the organism, culture is still difficult despite its lengthy history of use. Changes in pH or the use of proton pump inhibitors (PPIs) can indirectly affect distribution of *H. pylori* [14].

This study demonstrated that the sensitivity and specificity for culture method were 98.6% and 89.7% respectively. This is in agreement to Aftab et al. [15], They reported that, for *H. pylori* culture, the sensitivity and specificity were 92.1% and 100% respectively. The research done by Atkinson et al.[16] showed that this approach had sensitivity and specificity of 60% and 100% respectively. On the other hand, Tsuda et al. [10] noted a sensitivity of 67.9% and a specificity of 79.4%.

These different results in the studies can be explained by certain elements such as poor specimens quality, delay in transport, aerobic atmosphere exposure or unexperienced microbiologist [17].

This research revealed a rate of 1% for false negative cases by culture method. This may be due to *H. pylori* can present in two different forms, spiral form that is actively dividing and a coccoid form. Coccoid forms are identified as viable however non-culturable [17].

There are numerous stool antigen tests available commercially. Many studies have claimed that the SAT is beneficial for the initial diagnosis and post-treatment follow-up of *H. pylori* infection regardless of the variation in reported sensitivity and specificity rates. Most of them have acceptable results [8].

This study revealed that the stool antigen test yielded 90.1 % sensitivity and 82.8% specificity where they were relatively close to the research findings noticed by Miftahussurur and Yamaoka [5]. Also our results were in agreement to results of Hussein et al. [17] who reported that, sensitivity and specificity were 95% and 91.2%, respectively for SAT.

False-negative results can happen as a result of using a proton pump inhibitor or antibiotics and low bacterial load. The SAT, on the other hand, has commercially available variants that are not impacted by PPIs and do not require fasting. Additionally, numerous studies have

shown the efficiency of this technique in identifying infected patients during therapy and in assessing the cure of *H. pylori* infection [18].

In this study, ELISA showed 95.8 % sensitivity and 62.1 % specificity. The result is quite similar to what was noticed by Atkinson et al. [16], Amgalanbaatar et al.[19] and Hussein et al. [17.]

ELISA reported great sensitivity and low specificity when compared to PCR. When using a test to detect a severe but manageable illness, sensitivity is an important parameter. So, ELISA can be used as a first-line test for *H. pylori* diagnosis regardless of having low specificity. It is recomended that patients who were first positive with "high sensitivity/low specificity" tests have a second line-test which has"low sensitivity/high specificity" to appropriately diagnose illnesses. This will make it possible to determine that most false positives are actually disease-negative, and ELISA will also be safer, less impacted by errors of samples, and less burdensome for patients [20].

CONCLUSION

We concluded that, the bacteriological culture demonstrated the best overall performance followed by SAT and ELISA, in diagnosis of *H. pylori* when compared with PCR. And also that noninvasive ELiSA is highly sensitive test for first-line detection of *H. pylori* infection. A recommnded strategy for detecting the eradication of *H. pylori* in patients subjected to treatments is combining the findings of two or more methods.

The article had not been published or under consideration by another journal or any other reviewed media. No financial or non-financial conflict of interest have been declared by authors. All authors participated equally to the manuscript and approved the version submitted.

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