Ref: Ro J Infect Dis. 2024;27(2)
DOI: 10.37897/RJID.2024.2.11

Invasive methods for detection of *Helicobacter pylori* infection in gastric disorders patients undergoing endoscopy from Mosul city, Iraq

Muhammad Abdul-Ghani Muhammad, Anmar Ahmed Altaie, Bassima Ahmed Abdullah

Department of Biology, College of Science, University of Mosul, Mosul, Iraq

ABSTRACT

Objectives. The identification of *H. pylori* in Iraq lacks a standardized technique and there is limited information on the efficacy of different diagnostic methods. This research aims to determine the prevalence of H. pylori and evaluate the effectiveness of invasive techniques, namely the Rapid Urease Test (RUT), histopathological examination, and ure A gene by PCR, for detecting *H. pylori.*

Method. Gastric biopsies specimens were collected from 50 patients with gastric disorders who underwent endoscopy after meeting the inclusion criteria. Patients were classified into *H. pylori*-positive cases and negative cases by testing positive with at least two of the three testing methods, according to the case definition used in the study.

Outcomes. Of the 50 gastric biopsies, 58% were *H. pylori*-positive cases, 26% were H. pylori -negative cases, and the last 16% were indeterminate case according to cases definition. The ure A gene PCR was the most sensitive method, followed by histopathological examination and RUT testing, with sensitivities of 96.6%, 86.2% and 79.3% respectively. Histopathology examination was the most specific with a specificity of 95.2%, followed by ure A gene PCR with 85.7%, while RUT was the least specific with a specificity of 81%.

Conclusion. The current study revealed a moderate prevalence of *H. pylori* infection compared with previous studies. The sensitivity and specificity of the three invasive methods varied, and a combination of two methods may be necessary for a definitive diagnosis of *H. pylori* infection.

Keywords: *H. pylori,* gastric disorders patients, invasive methods

INTRODUCTION

Helicobacter pylori (H. pylori) is a Gram-negative bacterium that colonizes the mucus layer of the gastric epithelium of 50% of humans worldwide. It possesses several virulence factors that allow it to successfully survive in the stomach, including the ability to generate urease. This allows the bacteria to persist in the stomach for a long time. Infection with *H. pylori* can cause gastritis and gastric ulcer . Since it is classified as a type 1 carcinogen, if *H. pylori* remains in the body without being eliminated, it can lead to chronic gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer [1]. A variety of tests available for detecting H. pylori infection, some of which are non-invasive while others invasive. Non-invasive tests, such as urea breath test, serologic tests, stool antigen test, and PCR, are typically used for patients who do not need to undergo gastroscopy. Invasive methods, using endoscopy, include RUT, histology, culture, and PCR. Each test has its own pros and cons [2]. Although non-invasive methods of detecting H. pylori exist, culture remains the gold standard which requires competence and a lot of materials making it expensive [3]. *H. pylori* is a fastidious bacterium making it very delicate to culture. Molecular methods particularly PCR have been reported by several researchers with excellent sensitivity and specificity for detection *H. pylori* infection in gastric biopsies [4]. Test selection depends on availability, cost-effectiveness, Clinical status, preceding antibiotic and

Corresponding author:
Muhammad Abdul-Ghani Muhammad
E-mail: mohasbio117@uomosul.edu.iq

Article History:
Received: 15 May 2024
Accepted: 28 June 2024

proton pump inhibitor (PPI) therapy, occurrence of infection in the population, etc. [5]. For a city like Mosul, the rates of H. pylori infection are considered widespread and, with Lack of modern diagnostic options and financial resources, Choosing the suitable method for early and clear infection Detection can be vital and in many cases life-saving Situation. Therefore, our study focus on H. pylori prevalence and efficacy of the three different invasive techniques to diagnose H. pylori infection in Mosul city. These techniques included the RUT rapid test, histopathological examination, and ure A gene PCR.

METHODS

Study Population

A total of 50 patients were referred to the endoscopic units for one or more of the following symptoms: Epigastric pain, Dysphagia, Dyspepsia, Nausea and Vomiting, Weight loss, Bloating, Melena, and Bleeding at Ibn Sina Teaching Hospital, Researcher University Hospital and Al-Jumhuri Teaching Hospital were participated in this study from the period of March to December 2023. The study population consisted of 21 males and 29 females (ranging 15 to 79 years). Our study excluded patients who had been taking antibiotics or proton pump inhibitors for at least one week. Each individual provided informed consent before undergoing endoscopy. Five gastric biopsy specimens were collected using endoscopic forceps with aseptic precautions. One specimen was used for PyloPlus RUT, two for histopathology, and two for ure A gene PCR. Case definition for Positive H. pylori status was defined as a positive by at least two of the three testing methods PyloPlus RUT Test, ure A gene PCR and histopathology examination but a negative H. pylori status was confirmed when testing methods negative in all three tests while indeterminate H. pylori status were identified when Study participants testing positive in only one of the three testing methods.

PyloPlus Rapid Urease Test

By placing one freshly antral gastric biopsy in the test well, close and wait for the results. positive result in as little as 1 minute (change the color from yellow to pink or red), negative result (no change in color) in 1 hour [6]

DNA extraction and conventional PCR

Genomic DNA Extraction Kit (cat.no 10023- korea) was used for direct extracted bacterial DNA from two antral gastric biopsies. The DNA concentration and purity of each patient's sample were measured using the NanoDrop in accordance with the manufacturer's instructions for assessment the quality of DNA extraction and downstream application. The primers HPU1, 5'-GCCAATGGTAAATTAGTT-3'and HPU2, 5'-CTCCTTAATTGTTTTTAC-3' specific for ure A gene of *H. pylori* was used and the technique was performed in accordance with method of Clayton et al. [7]. Amplification of DNA was carried out in a final volume of 25 µl reaction mixture. The PCR condition of the amplification cycles consisted of as illustrated in Table 1. PCR products were separated using gel electrophoresis on 2% agarose and viewed under a UV transilluminator. Bands which were 411-bp in size, were considered as a positive result.

TABLE 1. The PCR condition of the amplification Cycles and Number of Cycles

Steps	Names	Temperature C°	Time (min.)	No. of cycles
1	Initial denaturation	94	7	1
2	Denaturation	94	0.45	35
3	Primer annealing	45	0.30	
4	Extension	72	0.30	
5	Final extension	72	7	1
5	Hold	4	15	

Histopathology Examination

Two biopsy samples from the antral region of the stomach were collected for examination under a microscope. The samples were preserved in a solution of 10% formalin and stained with hematoxylin and eosin. Giemsa staining was also used to verify the presence of *H. pylori* if the initial staining was inconclusive as that shown in (Figure 1).

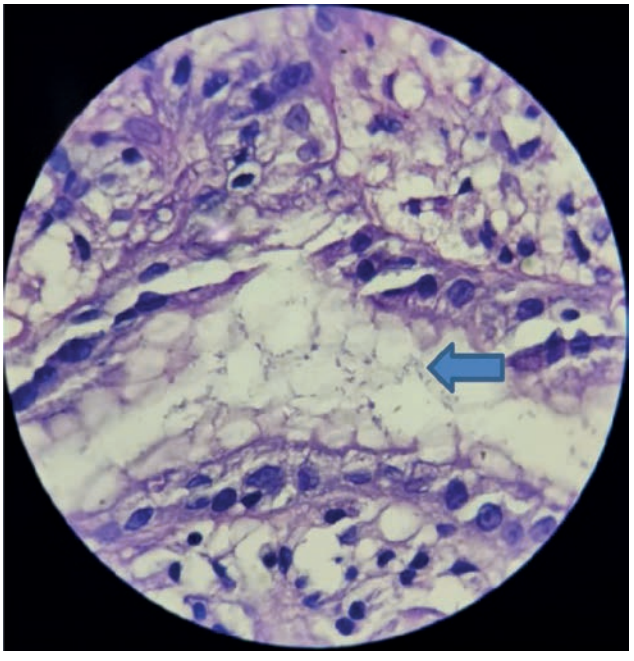

FIGURE 1. Histological section of stomach lining tissue viewing *H. pylori* on the surface of the mucosa (observed using Giemsa stain × 100)

Statistical analysis

The statistical analysis was conducted using SPSS software version 25.0. Sensitivities and specificities of various detection methods were determined depending on the defined cases of Helicobacter pylori positivity as the gold standard. crosstabs analyze is used to find the association between variables.

RESULTS

The endoscopic findings were gotten from a report by gastroenterologists related with 50 gastric disorder patients undergoing OGD endoscopy. The results indicate that gastritis (either pan gastritis or antrum gastritis) was the most common finding, observed in 19 patients (38%), while lax cardia was seen in only 2 patients (4%), as depicted in Figure 2.

A combination of at least two positive tests could detect 29 (58%) cases as positive, eight (16%) cases were classified as indeterminate because they tested positive in only one test, while all three; test could detect 13 (26%) case as negative (Table 2).

TABLE 2. Number and Percentage of *H. pylori* infection detection by RUT, histopathology and PCR tests

Name of positive methods	Number of samples (%)	Case defination (%)
RUT, histopathology and PCR	18(36)	29(58) Positive cases
RUT and histopathology	1 (2)	
RUT and PCR	4(8)	
Histopathology and PCR	6(12)	
Only RUT	4(8)	8(16) Indeterminate cases
Only histopathology	1(2)	
Only PCR	3(6)	
All three-test negative	13(26)	13(26) Negative cases
Total	50(100)	

The study population consisted of 21(42%) were males and 29(58%) were females with different age groups with a mean age of 38.9 ±18.05 years range from 15-79 years.

Distribution of age groups according to case definition show high prevalence of *H. pylori* infection among age groups 60≤ reach to 66.7% as shown in Table 3. The prevalence of *H. pylori* in males was found to be 11 (37.9%) while in females 18 (62.1%) according to case defination.

TABLE 3. Number and percentage of age groups according to case definition of *H. pylori* infection

Age	Case definition (%)	Negative	Positive	Total
15-29	Count	8	14	22
	%within Age	75%	25%	100%
30-44	Count	6	2	8
	%within Age	75%	25%	100%
45-59	Count	4	7	11
	%within Age	36.4%	63.6	100%
≥60	Count	3	6	9
	%within Age	33.3%	66.7%	100%
Total	Count	21	29	50
	%within Age	42%	58%	100%

Distribution of gastric disorders according to case definition show high prevalence of *H. pylori* infection among combined gastritis and gastric ulcers cases reach to 100% as shown in Table 4.

There are 27(54%) cases were positive to RUT Test out of 50 cases, it was found that highest number of RUT positive samples obtained from patient with combined gastritis and gastric ulcer 5/5 (100 %) as that shown in Table 5.

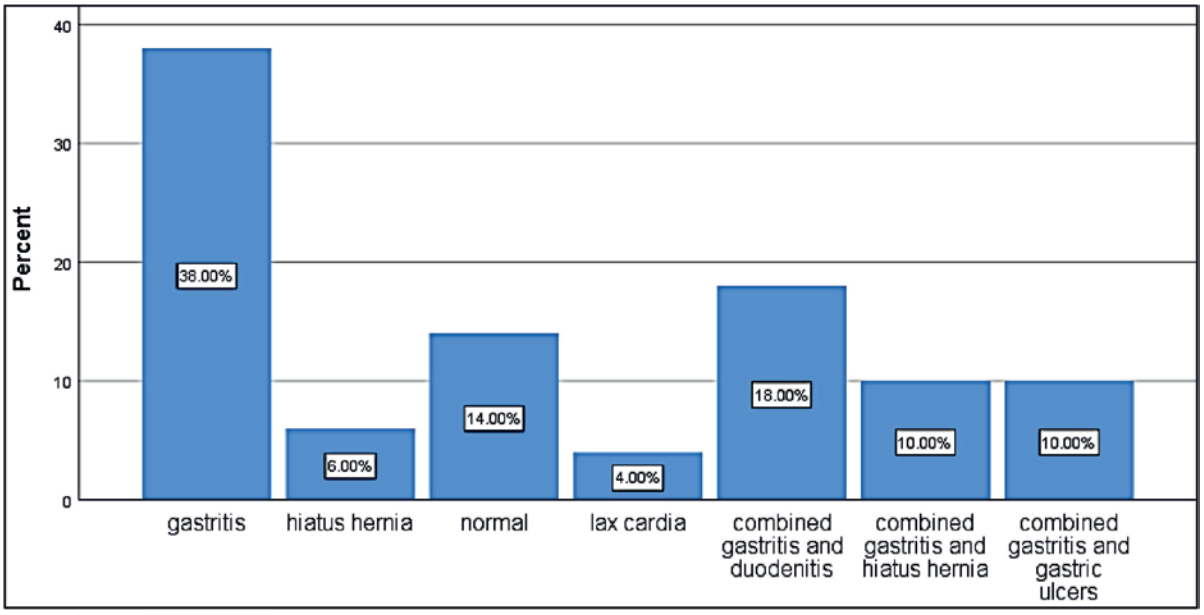

FIGURE 2. Percentage of Endoscopic Findings (n=50) under Study

TABLE 4. Distribution of gastric disorder according to case definition

Endoscopic finding	Case definition (%)	Negative	Positive	Total
Gastritis	Count	3	16	19
	%within endoscopic finding	15.8%	84.2%	100%
Hiatus hernia	Count	3	0	3
	%within endoscopic finding	100%	0%	100%
Normal	Count	7	0	7
	%within endoscopic finding	100%	0%	100%
Lax cardia	Count	2	0	2
	%within endoscopic finding	100%	0%	100%
Combined gastritis and duodenitis	Count	3	6	9
	%within endoscopic finding	33.3%	66.7%	100%
Compined gastritis and haitus herrnia	Count	3	2	5
	%within endoscopic finding	60%	40%	100%
Compined gastritis and gastric ulcer	Count	0	5	5
	%within endoscopic finding	0%	100%	100%
Total	Count	21	29	50
	%within Age	42%	58%	100%

TABLE 5. Diagnosis of *H. pylori* infection by RUT Test in patients with various gastric disorders

Endoscopic finding	RUT test	Negative	Positive	Total
Gastritis	Count	5	14	19
	%within endoscopic finding	26.3%	73.7%	100%
Hiatus hernia	Count	3	0	3
	%within endoscopic finding	100%	0%	100%
Normal	Count	7	0	7
	%within endoscopic finding	100%	0%	100%
Lax cardia	Count	2	0	2
	%within endoscopic finding	100%	0%	100%
Combined gastritis and duodenitis	Count	1	8	9
	%within endoscopic finding	11.1%	88.9%	100%
Compined gastritis and haitus herrnia	Count	5	0	5
	%within endoscopic finding	100%	0%	100%
Compined gastritis and gastric ulcer	Count	0	5	5
	%within endoscopic finding	0%	100%	100%
Total	Count	23	27	50
	%within Age	42%	58%	100%

There are 26 (52%) cases are positive to histopathology examination out of 50 cases, it was found that highest number of histopathology examination positive cases obtained from patient with gastritis 16/19 (84.2%) as that shown in Table 6.

TABLE 6. Diagnosis of *H. pylori* infection by histopathology examination in patients with various gastric disorders

Endoscopic finding	Histopathology examination	Negative	Positive	Total
Gastritis	Count	3	16	19
	%within endoscopic finding	15.8%	84.2 %	100%
Hiatus hernia	Count	3	0	3
	%within endoscopic finding	100%	0%	100%
Normal	Count	7	0	7
	%within endoscopic finding	100%	0%	100%
Lax cardia	Count	2	0	2
	%within endoscopic finding	100%	0%	100%
Combined gastritis and duodenitis	Count	4	5	9
	%within endoscopic finding	44.4%	55.6%	100%
Compined gastritis and haitus herrnia	Count	3	2	5
	%within endoscopic finding	60%	40%	100%
Compined gastritis and gastric ulcer	Count	2	3	5
	%within endoscopic finding	40%	60%	100%
Total	Count	24	26	50
	%within Age	48%	52%	100%

There are 31 (62%) samples are positive to ure A gene PCR out of 50 samples, it was found that highest number of ure A gene PCR positive samples obtained from patient with combined gastritis and gastric ulcer 5/5 (100%) as that shown in Table 7.

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of all three tests for identifying *H. pylori* infection were determined using positive cases as the gold standard, Table 8 shows the results.

DISCUSSION

Currently, there have been many different methods developed to detect the presence of *H. pylori*, it is still unclear which one is the most reliable gold standard. None of the diagnostic tests are foolproof or suitable for all situations, and each one has its own disadvantages. Despite the need for a fast, affordable, and accurate test in clinical settings, there is currently no single test that is considered the best for detection *H. pylori* infection [8,9].

TABLE 7. Diagnosis of *H. pylori* infection by ure A gene PCR in patients with various gastric disorders

Endoscopic finding	*ure A* gene PCR	Negative	Positive	Total
Gastritis	Count	4	15	19
	%within endoscopic finding	21.1%	78.9 %	100%
Hiatus hernia	Count	2	1	3
	%within endoscopic finding	75%	25%	100%
Normal	Count	5	2	7
	%within endoscopic finding	71.4%	28.6%	100%
Lax cardia	Count	2	0	2
	%within endoscopic finding	100%	0%	100%
Combined gastritis and duodenitis	Count	3	6	9
	%within endoscopic finding	33.3%	66.7%	100%
Compined gastritis and haitus herrnia	Count	3	2	5
	%within endoscopic finding	60%	40%	100%
Compined gastritis and gastric ulcer	Count	0	5	5
	%within endoscopic finding	0%	100%	100%
Total	Count	19	31	50
	%within Age	38%	62%	100%

TABLE 8. Sensitivity, specificity, PPV, NPV and accuracy of different tests for *H. pylori* infection

Tests	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
RUT	79.3	81	85.2	73.9	80
Histo-pathology	86.2	95.2	96.2	83.3	90
ure A gene PCR	96.6	85.7	90.3	94.7	92

The selection of diagnostic method should be based on clinical indication, what tests are locally available, and the costs involved, as well as patient preferences [10]. We conducted a study to assess the effectiveness of three alternative diagnostic tests for accurately determining a patient's *H. pylori* status that would be suitable for our city. Of the 50 cases conducted in this study, 29 (58%) tested positive for H. pylori, while 13 (26%) were negative for the bacteria. 8 (16%) samples showed indeterminate results. This rate is considered low when compared to other nearby Gulf countries, in countries like the United Arab Emirates and Kuwait, *H. pylori* was detected in 90.39% of 437and 96.6% of 204, respectively [11,12]. Similarly, comparison with other Arabic Asian countries showed that Yemen and Jordan also had higher rates of *H. pylori* in gastric biopsy specimens reach to 82.2% of 275 and 82% of 197 respectively [13,14].

Moreover, when the results of our study were compared to other Asian countries such as Iran and India, it was found that *H. pylori* infection was detected in 61.3% and 67.1% of samples, respectively [15,16]. There are several factors that explain such differences in findings. For instance, the rates of *H. pylori* infection differ in various regions due to factors like the socioeconomic status of individuals, the methods used to detect the infection, and potential errors in gastric biopsy sampling [8,9]. Additionally, the uneven distribution of pathogens within gastric lining could also aide to the lower identification percentage of the organism in our study. Also, studies conducted in Asia and the Middle East have noticed a decrease in the detection of *H. pylori* infection, which can be attributed to the better hygienic and socioeconomic conditions in that particular region [17-19].

Our study according to case definition revealed, *H. pylori* infection was more common in ≥60 years aged group, followed by 45-59 years and 15-29 years aged groups, while *H. pylori* infection was shown to be less common among 15-29 age group this result disagreement with other studies [18,19] while agreement with others study [20]. The higher prevalence of infection in older age individuals is believed to be due to continuous exposure to pathogens throughout their adulthood [21]. Explanations for the variation in *H. pylori* infection rates among different age groups may be due to varying risk factors between adults and children. The increase in prevalence among adults may also be largely attributed to a cohort effect. In this study, the rate of *H. pylori* prevalence was higher in females at 62.1% compared to males at 37.9%. Despite previous research showing no gender difference in *H. pylori* prevalence, some studies have suggested a higher rate in males [22] and a greater likelihood of females developing gastric cancers after *H. pylori* infection [23]. The high prevalence of *H. pylori* in females in this study could potentially be linked to iron deficiency anemia, which is more commonly seen in females.

In our research, out of 50 samples, the RUT test showed positive results in 27 cases (54%). Other studies have also reported similar results, with RUT detecting the bacteria in 54.05% and 53.3% of cases, respectively. [24,25]. On the other hand, in previous study conducted in Iraq that found higher rate of H. pylori positive by RUT reach 87(82.08%) out of 106 cases [26]. Various factors can influence the outcome of RUT, such as the condition of the biopsy and the type of disease being tested. The accuracy of RUT is influenced by factors like the location, quantity, size, and bacterial concentration in the biopsy sample [23]. Combining biopsies from both the antrum and corpus before performing RUT can improve the test's sensitivity. However, it was found that the sensitivity of RUT decreases in patients with bleeding peptic ulcers [27]. Our research showed four false

positive outcomes, possibly caused by the presence of other organisms containing urease [28]. Out of the cases we examined, 6 (12%) tested positive for PCR and histology but negative for the RUT. The formalin in the biopsy forceps may have caused false negative results [29]. Also, a small gastric biopsy with only a few organisms present may result in a negative RUT, as at least 105 bacteria copies are needed for a positive result [30]. Furthermore, the coccoid form of H. Pylori has decreased urease activity, leading to negative test results [31].

In our research, *H. pylori* infection was found in 52% of cases through histological examination. Other studies have reported slightly different results, with some finding 55.6% and 54% of cases positive for H. pylori using histology in gastric biopsy samples [32,33]. In contrast, some studies have shown lower prevalence rates in 7.2% and 16.8% of cases [34,35], while others have reported higher rates of in 86.2% and 72.86% of cases [36,37]. In our study, the ure A gene PCR was detected in 62% of cases. Another study in Iraq found a similar rate in 52.5% of *H. pylori* cases detected using ure A gene PCR [38]. However, other studies have shown higher prevalence rates in 89.8% and 73.9% of cases than what we found in our research [39,40].

The sensitivity and specificity of three invasive tests for *H. pylori* diagnosis varied across the studies. Specificity was found 81% in our study, which is close to findings from other studies 78.5% and 75.6% [41,42] while the sensitivity of RUT was found (79.3%), compared to 96% and 95%. In other studies, [41,43].

Our research showed a sensitivity of 96.9% and specificity of 85.7% using ure A gene PCR. An Iranian study reported slightly different results, with sensitivity at 93.5% and specificity at 95.6% [43]. The ure A gene PCR test had the highest accuracy rate of 92%, as molecular methods are known for their rapid results and are less affected by sample transportation conditions. Only 1 case (2%) in our study tested negative for the ure A gene PCR test but positive in the other two tests. This is because the colonization of *H. pylori* in the stomach lining occurs in patchy manner, so taking a gastric biopsy sample that doesn't have any bacteria or fewer than 50 bacteria can lead to false results in PCR testing [44].

In our study, we used three different invasive tests, the most specific test was histological examination, with a specificity rate of 95.2% and a sensitivity rate of 86.2%. The sensitivity and reliability of the histological examination is influenced by various factors, such as the quantity and location of gastric biopsy specimens obtained [45]. Ideally, four specimens should be collected, with two from the antrum of the gastric lining and two from the corpus region. However, in our study, we only used two antral gastric biopsy specimens for histological examination, which may have affected the accuracy of the test. Furthermore, previous studies have shown significant differences in results between observers, indicating that the skills of the pathologist are crucial for diagnosis *H. pylori* [46,47]

CONCLUSION

The current study showed that the prevalence rate of *H. pylori* infection was lower compared to rates in other developing countries. Females and older age individuals had the highest prevalence of this infection. Detection of ure A gene directly from biopsy specimen helps in rapid detection of *H. pylori* infections. RUT had lower sensitivity and specificity, while histology was the most specific test and ure A gene PCR was the most sensitive. The accuracy of the three methods for detecting *H. pylori* can be ranked as follows: PCR > histology > RUT.

Conflict of interest: All authors declare there is no conflict of interest

Financial support: self-funding

Ethical of approval: The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee in accordance with document number (13874/22-3-2023) in order to obtain approval.

Acknowledgment: Thanks and appreciation to the University of Mosul and the College of Science for providing assistance to complete this research.

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