Evaluation of Noninvasive Versus Invasive Techniques for the Diagnosis of *Helicobacter pylori* Infection

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Background: *Helicobacter pylori* is one of the most common bacterial strains causing chronic infections, affecting over one half of the world's population. There is increasing interest in noninvasive methods for diagnosing *H. pylori* infection. The aim of the study was to evaluate 3 different noninvasive methods of diagnosis: the stool antigen test (HpSA), the serum antibody test, and the stool-polymerase chain reaction (PCR) test as against invasive methods based on histopathologic diagnosis.

Materials and Methods: Gastric biopsies were obtained during endoscopy. Sections were stained with hematoxylin and eosin and Giemsa stain. Serum samples were tested for *H. pylori* antibody using an enzyme-linked immnunosorbent assay kit for the semiquantitative determination of IgG antibodies; stool samples were tested for *H. pylori* antigen using polyclonal enzyme-linked immnunosorbent assay kits. DNA samples from stool specimens were extracted, followed by PCR for the detection of *H. pylori UreA*.

Results: The results revealed that 18/19 (94.7%) patients were positive for *H. pylori* infection as detected by Giemsa stain, and 84.2% were positive on the basis of hematoxylin and eosin stain, with a sensitivity and specificity of 88.9% and 100%, respectively. Diagnosis by noninvasive methods, including the serum antibody test, revealed a sensitivity and positive predictive value of 88.9% and 94.2%, respectively, whereas the stool antigen test recorded a sensitivity and positive predictive value of 72.2% and 92.9%, respectively. The stool-PCR test recorded a sensitivity of 72.2% and specificity of 100%.

Conclusions: Among the noninvasive methods for diagnosis of *H. pylori* infection, the 3 methods used in this study recorded promising results, including good sensitivity, which was the highest in the serum antibody test, whereas the stool-PCR test recorded excellent specificity.

Key Words: *H. pylori*, Giemsa, stool-PCR, stool antigen, ELISA

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Helicobacter pylori infection is known to play a causative role in various gastroduodenal diseases, such as gastritis and peptic ulcer.[1]

Infection with *H. pylori* triggers various malignant diseases of the stomach by inducing chronic inflammation of the gastric mucosa, which progresses further through the premalignant stages of gastric atrophy, intestinal metaplasia, dysplasia, and finally to gastric adenocarcinoma.[2,3] Epidemiological, molecular, biological, and experimental data clearly indicate that *H. pylori* plays a decisive role in the development and progression of gastric mucosa–associated lymphoid tissue (MALT) lymphoma,[4–6] which is why *H. pylori* infection is now considered a model for chronic bacterial infections causing cancer[7] and was classified by the World Health Organization and the International Agency for Research on Cancer in 1994 as a group 1 carcinogen in humans.[8]

Epidemiologic studies showed that *H. pylori* affects over 50% of the world's population, infecting 40% to 50% of the population in industrialized countries and 70% to 90% in developing countries.[9]

Accurate diagnosis is essential for the effective treatment and management of *H. pylori* infection. Several invasive and noninvasive diagnostic tests are used for the detection of *H. pylori* infection.[10] Invasive tests include endoscopy with gastric biopsy specimens, whereas noninvasive tests include serology, the urea breath test, the stool antigen test, and the polymerase chain reaction test (PCR). The histologic examination is dependent on the experience and accuracy of the observer but has the advantage of being able to determine the severity of the gastritis.[11] Various special stains have been devised to detect *H. pylori* in these histologic sections, but their specificity and sensitivity vary greatly.

In contrast, diagnosis of *H. pylori* infection by molecular methods—in particular, PCR—has the potential to detect more cases of infection because of greater sensitivity; however, these methods are technically demanding.[12] The culture and identification of the organism is time-consuming but advantageous in that antibiotic susceptibility tests can be conducted. The rapid urease test is a simple method for detecting *H. pylori* in gastric biopsy specimens, but some authors have indicated that it is unreliable in certain settings.[13]

Nowadays, there is increasing interest in noninvasive methods to diagnose *H. pylori* infection.[10] Antigen detection in stool is an attractive noninvasive

method that seems very suitable for clinical and epidemiologic studies as it can diagnose active infection in human fecal samples.[10] Assessment of the gastrointestinal tract (GIT) at endoscopy, combined with histologic examination of biopsy samples, yields a more complete clinical picture and, for this reason, it is unlikely to be totally replaced by noninvasive tests. Several methods are used to detect *H. pylori* in gastric biopsy specimens, such as the urease test, PCR on tissue specimens, and the *Campylobacter*-like organism test, but it has been difficult to establish a gold standard.[14,11]

PATIENTS AND METHODS

This study was carried out at the National Cancer Institute (NCI), Virology and Immunology Unit, Cancer Biology Department, Cairo University Endoscopy Unit, and Pathology Unit during the period from June 2009 through to February 2010. All attending patients were suffering from gastrointestinal disorders such as dyspepsia, gastritis, gastric ulcer, epigastric pain, vomiting, and gastroesophageal reflux disease, and we included those who agreed to participate in this study. Blood and stool samples were collected from 52 patients, but pathologic data were available for only 19 patients; the other 33 patients were excluded from our study, as their pathologic data were not available.

Data on clinical history including age, sex, patient's complaint, endoscopic findings, pathologic findings, and routine laboratory investigations (complete blood profile testing, differential blood count, liver and kidney functions, and abdominal computed tomography) were obtained for all patients from their files.

Stool and blood samples and multiple gastric biopsy samples from the cardia, fundus, and antrum were obtained from all patients. Stool (0.5 g) was collected and stored at −20°C until use. Venous blood (5 mL) was collected for serum separation and stored at −20°C until use. Biopsy specimens were taken during the endoscopy process, and Gram staining was carried out to detect *H. pylori* directly.

Histopathologic Evaluation of *H. pylori*

The tissue biopsies were fixed in formalin and embedded in paraffin. Sections were cut using a microtome at 4 μm thickness and mounted on glass slides. Paraffin sections were placed in an oven at 60°C for 15 minutes and then in xylene overnight for deparaffinization. The sections were rehydrated through 100% ethanol, two changes, 5 min each and 95% ethanol, two changes 5 min each and rinsed in distilled water. One slide was stained with hematoxylin and eosin (H&E), and another slide was prepared from each block and stained with Giemsa (1 g Giemsa, 60 mL methyl alcohol, 4 mL glycerol). Routine histopathologic examination was carried out to determine the exact histopathologic diagnosis.

In gastric biopsies diagnosed as carcinoma or lymphoma, detection of *H. pylori* was achieved by examination of adjacent unaffected gastric mucosa, including identification of associated gastric pathology.

Serological Diagnosis

Semiquantitative detection of *H. pylori* IgG antibodies in human serum was carried out in all patients using the RIDASCREEN Helicobacter enzyme immunoassay kit (R-Biopharm, Germany). The test was carried out as described by the manufacturer's instructions.

All patients were also tested for *H. pylori* with the stool antigen test (HpSA) using enzyme-linked immnunosorbent assay (ELISA) kits provided by Immunodiagnostik AG (Germany). The test was carried out as described by the manufacturer's instructions.

Molecular Diagnosis

Isolation of DNA from stool samples was performed using the Qiagen QIAamp DNA Stool Mini kit, Germany. Stool typically contains many compounds that can degrade DNA and inhibit downstream enzymatic reactions. To ensure removal of these substances, the QIAamp DNA Stool Mini Kit contains InhibitEX Tablets that efficiently adsorb these substances early in the purification process so that they can be removed easily by a quick centrifugation step carried out according to the manufacturer's instructions. In addition, the kit contains buffer ASL, which has been especially developed to remove inhibitory substances from stool samples. The 2 stool samples seeded with *H. pylori* as positive controls were processed with the other stool samples.

PCR was carried out on the extracted DNA using the PrimerDesign ingene Kit for *H. pylori*, Germany, which used *UreA* gene as a primer; the primers have 100% homology with all reference sequences in the NCBI database. The kit contained an *H. pylori* template, which was used as a positive control, and an endogenous ACTB primer (β-actin primer).

Each DNA sample was exposed to PCR for the β-actin gene using the ACTB primer supplied with the kit to assess its quality and integrity for further run for the *UreA* gene specified for *H. pylori*.

Samples were run simultaneously with 2 positive controls (the positive control template supplied with the kit and the DNA extracted from the sample previously seeded with *H. pylori*). Negative control reaction with distilled water was performed with each batch of amplification to exclude the possibility of contamination.

Thermal cycling was performed using a T-Gradient thermal cycler (Biometra, Germany). The thermal cycling parameters were as follows: an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes. All PCR products were analyzed by gel electrophoresis in a 2% (wt/vol) agarose gel in 1 × Tris-acetate-EDTA buffer.

RESULTS

This study comprised 19 patients: 13 were male (68.4%) and 6 were female (31.6%). The patients' ages ranged from 29 to 76 years with a mean of 47.3 ± 13.13 years.

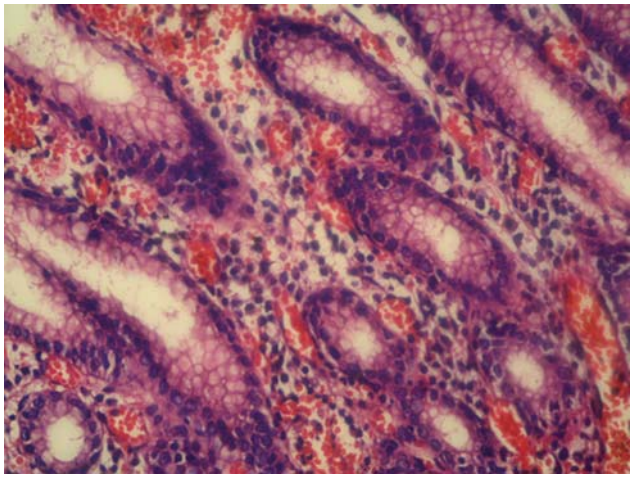

FIGURE 1. Gastric mucosal glands with many intraluminal *Helicobacter pylori* (hematoxylin and eosin: ×400).

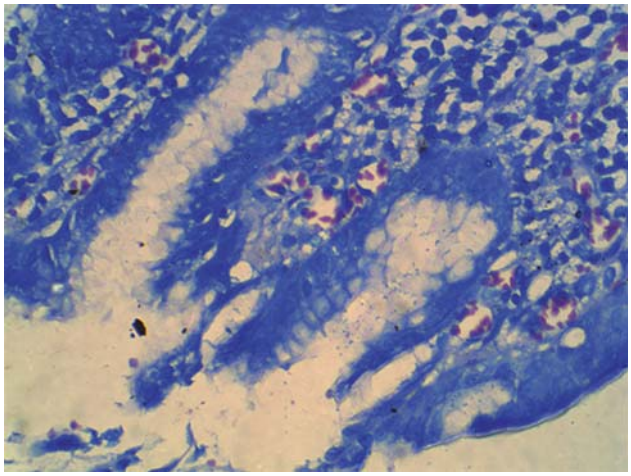

FIGURE 3. Giemsa stain showing gastric mucosa with many intraluminal *Helicobacter pylori* (×400). full color online

Diagnosis of the 19 patients revealed 9 cases of gastric lymphoma and 5 cases of adenocarcinoma; all of which had associated chronic gastritis. One patient with esophageal cancer, 1 with hepatocellular carcinoma, and 3 other patients were also confirmed to have chronic gastritis. Atrophic gastritis was detected in 9 patients and activity in 2; intestinal metaplasia was detected in 5 biopsies.

Detection of *H. pylori* infection was evaluated using 2 stains: H&E and Giemsa; the last one was used as the reference method of diagnosis (Figs. 1–3). Eighteen patients (94.7%) were infected with *H. pylori* detected by Giemsa stain (true positive).

In our study, all male patients (13/13; 100%) were positive for *H. pylori* by histopathologic examination, whereas 5/6 (83.3%) female patients were positive; in addition, all urban patients (11/11; 100%) and rural patients 7/8 (87.5%) were positive for *H. pylori* infection. Our results also revealed that all patients suffering from gastric cancer (adenocarcinoma and non-Hodgkin lymphoma) (14/14; 100%) were infected with *H. pylori*. All patients suffering from gastritis associated with intestinal metaplasia were positive for *H. pylori*. Also, the majority of patients with atrophic gastritis (8/9) were positive for *H. pylori*. All patients suffering from vomiting, (8 patients) ulcer, (6 patients) and dyspepsia (3 patients) were positive for *H. pylori* (Table 1).

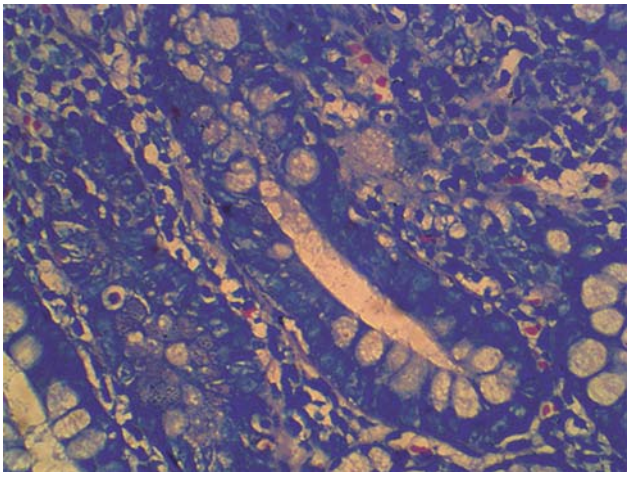

FIGURE 2. Giemsa stain showing gastric glands with a few intraluminal *Helicobacter pylori* (×400). full color online

On the basis of H&E stain, 16 (84.2%) patients were positive for *H. pylori* infection; all of them were also positive for Giemsa stain (true positive). There were no false-positive results; 3 cases were negative, one of which was true negative, whereas the other 2 were false negative, recording a sensitivity of 88.9%, specificity of 100%, positive predictive value (PPV) of 100%, and negative predictive value (NPV) of 33.3% for H&E stain (Tables 2, 3).

Serological diagnosis using the stool antigen test revealed that 14 patients (73.7%) were positive for *H. pylori*, of whom only 1 showed a false-positive result. Five patients were negative for *H. pylori* by the stool antigen test, whereas they were positive by Giemsa staining (false negative), recording a sensitivity of 72.2% and PPV of 92.9%. In contrast, there was no true-negative result, and therefore specificity could not be calculated (Tables 2, 3).

On the basis of the serum antibody test, 17/19 patients (89.5%) were positive; 16 of them were also positive by Giemsa stain (true positive) and 1 case was negative (false positive), recording a sensitivity of 88.9% and PPV of 94.2%; the remaining 2 cases, which were negative by serum antibody, were positive by Giemsa staining (false negative) (Tables 2, 3).

Diagnosis based on PCR analysis of a stool sample revealed that 14/19 (73.7%) cases were positive (Fig. 4), all of which were true positive. Five of 19 (26.3%) cases were negative, of which only 1 case was true negative, recording a sensitivity of 72.2%, specificity of 100%, PPV of 92.9%, and NPV of 16.6% (Tables 2, 3).

TABLE 1. Prevalence of *Helicobacter pylori* According to Different Diagnoses and Symptoms

Diagnosis	*H. pylori* Positive by Histopathology (Giemsa Stain) (n = 18)	Male (n = 13)	Female (n = 6)	Urban (n = 11)	Rural (n = 8)
Gastric lymphoma (n = 9) and adenocarcinoma (n = 5)	14/14 (100%)	9/9 (100%)	5/5 (100%)	8/8 (100%)	6/6 (100%)
Esophageal (n = 1) and hepatocellular cancer (n = 1)	2/2 (100%)	2/2 (100%)	0/0	2/2 (100%)	0/0
Gastritis (n = 19)	18/19 (94.7%)	13/13 (100%)	5/6 (83,3%)	11/11 (100%)	7/8 (87.5%)
Symptoms					
Dyspepsia (n = 3)	3 (100%)	2/2 (100%)	1/1 (100%)	2/2 (100%)	1/1 (100%)
Vomiting (n = 8)	8 (100%)	5/5 (100%)	3/3 (100%)	4/4 (100%)	4/4 (100%)
Epigastric pain (n = 8)	7 (87.5%)	4/4 (100%)	3/4 (75%)	5/5 (100%)	2/3 (66.6%)
Gastric ulcer (n = 6)	6 (100%)	4/4 (100%)	2/2 (100%)	4/4 (100%)	2/2 (100%)

DISCUSSION

Infection with *H. pylori* is considered one of the most common chronic bacterial infections throughout the world; over one half of the world's population is infected with this organism.[15] As its cure prevents peptic ulcer disease, chronic gastritis, and possibly MALT lymphoma, the importance of accurate and rapid methods for detection of this pathogen is hardly overestimated.[16] *H. pylori* eradication leads to regression and prevents the progression of precancerous lesions. Eradication therapy may be used in high-risk populations to reduce gastric cancer incidence; it can reverse many biochemical, genetic, and epigenetic changes that *H. pylori* infection induces in the stomach.[2]

Currently available tests for the diagnosis of *H. pylori* infection have relatively high sensitivities and specificities, but each has its limitations in clinical application.[17]

At present, there is an increasing interest in noninvasive methods to diagnose *H. pylori* infection; indeed, they can profitably replace endoscopy in predicting the diagnosis.[10]

The objective of this study was to evaluate the efficiency of noninvasive methods for the diagnosis of *H. pylori* infection in comparison with a histopathologic method (using Giemsa stain on gastric biopsies) as the gold standard.

We examined 3 different noninvasive methods for diagnosis: the serological method depending on detection of IgG against *H. pylori* in serum using the ELISA test; the stool antigen test for detection of *H. pylori* antigen in feces (HpSA test); and the stool-PCR test for detection of *UreaseA* DNA of *H. pylori*. All 3 methods were compared with the histopathologic diagnosis.

We detected *H. pylori* infection in 97.4% of our studied sample using Giemsa staining. This figure agrees with data reported by Frenck and Clemens,[18] who reported that in Egypt *H. pylori* infection was seen in 50% of children aged 3 years and in 90% of the adult population.

Bassily et al[19] recorded that in a rural area in Egypt 13% of the children aged 7 to 9 months were positive for IgG antibodies against *H. pylori* urease, using a commercial ELISA test, and that by 18 months of age seropositivity had increased to 25%, whereas 88% of the mothers were positive using the same assay. Interestingly, in the same study, Bassily and colleagues reported that increased education was significantly associated with an increased risk for infection among mothers, which points strongly to the importance of mass hygiene education to change the traditional inherent fallacious hygiene practice.

The prevalence of *H. pylori* infection in Egypt is similar to those of other countries in the Middle East; for example, in 1 study in Libya, the prevalence of *H. pylori* infection was 50% of the population between the ages of 1 and 9 years, 84% between 10 and 19 years, and 94% in adults.[20] In Saudi Arabia, 40% of the children aged between 5 and 9 years were infected with *H. pylori*, whereas 80% were found to be infected among adults.[21]

In the African population, the incidence is nearly similar; for example, in Ethiopia, the prevalence of infection was 48% between the ages of 2 and 4 years, whereas >95% of the adults were found to be infected.[22]

TABLE 2. Detection of *Helicobacter pylori* by Different Methods Used in the Study

	Giemsa Stain	Hematoxylin and Eosin	Serum Antibody	Stool Antigen	Polymerase Chain Reaction–Stool
Positive	18 (94.7%)	16 (84.2%)	17 (89.5%)	14 (73.7%)	14 (73.7%)
True positive	18 (100%)	16 (100%)	16 (94.1%)	13 (92.9%)	14 (100%)
False positive	0	0	1 (5.9%)	1 (7.1%)	0
Negative	1 (5.3%)	3 (15.8%)	2 (10.5%)	5 (26.3%)	5 (26.3%)
True negative	1 (100%)	1 (33.3%)	0	0	1 (20%)
False negative	0	2 (66.7%)	2 (100%)	5 (100%)	4 (80%)

TABLE 3. Sensitivity and Specificity of Different Methods Used for Diagnosis of *Helicobacter pylori*

Different Methods of Diagnosis	Hematoxylin and Eosin Stain (%)	Stool Antigen Test (%)	Serum Antibody Test (%)	PCR for Stool (%)
Sensitivity	88.9	72.2	88.9	72.2
Specificity*	100	—	—	100
PPV	100	92.9	94.2	92.9
NPV	33.3	—	—	16.6

*Specificity could not be calculated in stool antigen and serum antibody because there were no true-negative cases.
NPV indicates negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value.

In Nigeria, 82% of the population between 5 and 9 years and 91% of the adult population were found to be infected with *H. pylori*.[23]

A high prevalence of *H. pylori* infection has also been observed in India both in adults (88%)[24] and in children younger than 5 years (57%).[25]

These figures actually support the well-known fact that the prevalence of *H. pylori* infection increases with advancing age and is higher in developing countries and among low socioeconomic populations, probably because of conditions that favor the infection, such as poor hygiene, crowded living conditions, and inadequate sanitation.[26]

The last statement gains more strength when retrieving data reporting *H. pylori* infection from developed countries; for example, in Sweden, only 11% of the adults (25 to 50 y) were reported to be infected with *H. pylori*,[27] whereas in the United states 20% of the adult population was infected.[28]

Primary gastric non-Hodgkin lymphoma represents more than half of all primary gastrointestinal non-Hodgkin lymphoma cases in the western world, and an increasing incidence has been reported.[5]

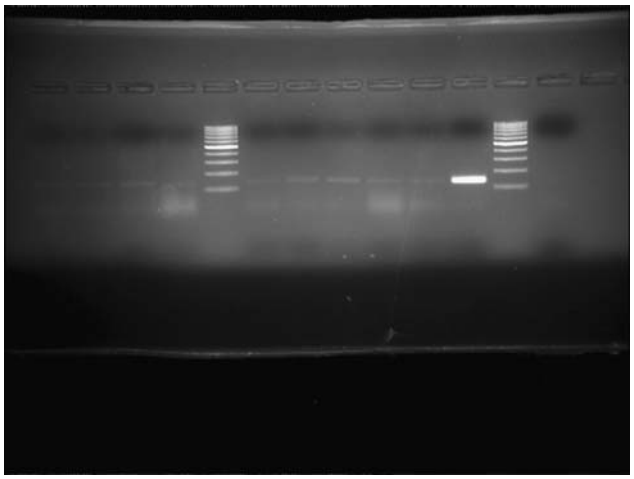

FIGURE 4. *Helicobacter pylori* stool-polymerase chain reaction amplicon visualized on 2% agarose gel. M: 100 bp DNA ladder lanes 5 and 12. Lanes 10 and 11: positive control; 13: negative control; all lanes were positive except lane 14 (expected product size is 150 bp).

Unfortunately, in Egypt we lack a well-documented population-based cancer registry to compare the overall incidence of primary gastric lymphoma in the Egyptian population with other worldwide population-based cancer registries.

In our study, almost half (9/19; 47.4%) of the *H. pylori*–positive individuals had primary gastric non-Hodgkin lymphoma.

In a review of extranodal lymphoma cases among Egyptian patients who had presented to the Department of Pathology, NCI, Cairo University, during the period 1987 to 1998, 381 cases of lymphoma were diagnosed; 50% were located in the GIT, constituting 5.6% of all malignant lymphomas diagnosed during the same period, of which the stomach represented the most common site, representing 49% of cases.[29] In a study conducted by Ismail,[30] who studied the profile of primary gastrointestinal lymphoma in Egyptian patients referred to the NCI, Cairo University, from 1998 to 2000, primary gastrointestinal lymphoma constituted 7% of the total primary gastrointestinal malignancies, of which primary gastric non-Hodgkin lymphoma constituted 38% of the cases and 9% of the primary gastric malignancies. In the latest registry of the NCI, Cairo University, Egypt, for the period 2003 to 2004, the stomach was the most common site for extranodal lymphoma in the GIT, constituting 37.2% of all gastrointestinal lymphomas and 19.2% of all gastric malignancies.[31] In a series on 208 Egyptian patients with primary gastrointestinal lymphomas, the stomach was the main site of involvement, constituting approximately 75% of cases, a figure clearly higher than in many other published series.[32] For example, in an epidemiologic western study conducted in the United States, primary gastric MALT lymphoma represented approximately 12% of the extranodal non-Hodgkin lymphomas that occurred among men and approximately 18% of extranodal non-Hodgkin lymphomas among women, and during the period 1999 to 2003 the annual incidence in the United States was about 1 case for every 100,000 individuals in the population.[33] Also in a population-based study in Germany and over a period of 3 years, 94 patients with primary gastric lymphoma were recorded out of a total population of 3.5 million. The standardized incidence rates in Saarland and Franconia were 0.7 and 0.8 cases per 100,000, respectively.[34]

It is noteworthy to highlight the results of a recent study focusing on coinfection with hepatitis C virus

(HCV) and *H. pylori*, in which Furusyo et al[35] reported that *H. pylori* infection rate was significantly higher for HCV-infected patients (67 of 76, 88.2%) than for HCV-noninfected controls (158 of 228, 69.3%). Endoscopic findings showed that the rates of gastric ulcers and gastritis were significantly higher for the 67 HCV-infected patients with *H. pylori* infection (34.3% and 77.6%) than for the 158 HCV-noninfected controls with *H. pylori* infection (15.2% and 57.6%).[35] In an Egyptian study, El-Masry et al[36] reported that *H. pylori* positivity increased significantly ($P = 0.03$) in HCV-infected patients when compared with healthy controls: *H. pylori* infection was found in 50 (55.6%) of 90 HCV-infected patients versus 26 (39.4%) of 66 healthy controls. In HCV-infected patients, the prevalence of *H. pylori* infection increased significantly ($P = 0.04$) from chronic active hepatitis to cirrhosis. Because Egypt has one of the highest prevalence rates of HCV infection in the world with different strains involved,[37] the diagnosis and eradication of *H. pylori* infection is of paramount importance not only because of the gastric sequel of *H. pylori* but also because treatment for *H. pylori* infection may prove beneficial in those patients with chronic hepatitis C.

In the current study, the Giemsa stain was selected as the reference method for the diagnosis of *H. pylori* infection because of its high sensitivity and specificity. Laine et al[38] tested the sensitivity and specificity of H&E, Geimsa, and Genta stains for the detection of *H. pylori* infection. They reported that the Giemsa stain seemed to be the preferred stain for *H. pylori* diagnosis because of its good sensitivity, excellent specificity, and lack of technical difficulty in preparation.[38]

In this study, the H&E stain failed to detect 2 cases of *H. pylori* infection diagnosed as positive by the Geimsa stain (false negative), whereas there were no false-positive results, recording a sensitivity of 88.9% and a specificity of 100%.

Kacar et al[39] demonstrated that the low sensitivity of the H&E stain is probably because of the lack of contrast between the bacteria and the surrounding tissues, whereas Rotimi et al[40] stated that the specificity of the H&E stain is also low because of its nonspecific staining of the non-*H. pylori* bacteria resident in the stomach. Kacar et al[39] demonstrated that modified Giemsa is an inexpensive and easily applicable stain that can be used in 15 minutes. The lack of contrast is a disadvantage of the Giemsa technique, but careful observation should allow identification of the organisms. Although they found the highest sensitivity and specificity of *H. pylori* detection by immunohistochemistry, they finally concluded that the Giemsa stain is the best stain for the detection of *H. pylori* because of its low cost, short hands-on time required for staining, and very high sensitivity and specificity combined with a high interobserver agreement. In addition, Rotimi et al[40] finally concluded that the modified Giemsa stain is the method of choice, because it is sensitive, inexpensive, easy to perform, and reproducible.

In this study, infection with *H. pylori* was detected in 18/19 patients (94.7%) diagnosed histologically using the Giemsa stain, 16/19 (84.2%) by H&E stain, 17/19 (89.5%) by the serum antibody test, and 14/19 (73.7%) by both the stool antigen test (HpSA) and the stool-PCR test. Abdel-Haq[41] recorded a lower frequency of *H. pylori* infection (78%) using the serum antibody test among a group of pediatric patients undergoing endoscopy. In contrast, our results were higher than those obtained by Abdel-Wadood and Batarfy,[42] who recorded a frequency of *H. pylori* infection in Egypt of 61% by serodiagnosis, which was lower than that obtained in this study; however, the stool antigen test result was in accordance with our results, recording 76% prevalence among a studied group of adult patients with dyspepsia undergoing GIT endoscopy. The high frequency of *H. pylori* infection in this study could be because the patients attending the NCI are in poor condition and suffer from severe symptoms.

The sensitivity and PPV of the serum antibody test for detecting *H. pylori* infection, compared with histologic examination of gastric biopsies using the Giemsa stain, showed high sensitivity of 88.9% and PPV of 94.2%. This was in accordance with the review conducted by Laheij et al,[43] in which a total of 36 different commercially available *H. pylori* serology kits were evaluated on 26,812 patients, and the mean sensitivity for *H. pylori* serology was reported as 92% (85% to 96%). Abdel-Haq[41] and Abdel-Wadood and Batarfy[42] conducted a similar study in Egypt to evaluate the serum antibody test and recorded sensitivity values of 82.4% and 82.3%, respectively, which were lower than the values obtained in this study.

The main disadvantage of the serum antibody test is that it cannot distinguish between active and previous infections, as the antibodies persist in blood for a long period of time, leading to false-positive results.[44] Hung et al,[45] through a quantitative ELISA test, reported a high sensitivity of 100.0% for detection of *H. pylori* infection in patients with atrophic gastritis versus 96.5% in patients without atrophic gastritis. Hung et al[45] therefore concluded that a quantitative ELISA test is suitable for the diagnosis of *H. pylori* infection in patients with atrophic gastritis because of its excellent sensitivity.

In the current study, the stool antigen test showed a sensitivity and PPV of 72.2% and 92.9%, respectively. These values were higher than those obtained by Rafeey and Nikvash,[46] who recorded a sensitivity and PPV of 54.8% and 82.9%, respectively, for the stool antigen test (HpSA) compared with histolopathologic analysis of gastric biopsies carried out on a group of children with dyspeptic symptoms. In contrast, Emara et al[47] in a prospective multicenter study conducted throughout Egypt on patients undergoing gastroscopy to evaluate HpSA against histology and urease tests reported a sensitivity and PPV of 96.8% and 96.4%, respectively. Bashaar and Hashem[44] also recorded a higher sensitivity and PPV compared with our findings using the stool antigen test as against endoscopy-based tests in a study conducted on a group of patients complaining of chronic dyspepsia, whereas the serum antibody test results were in accordance with ours. In a study conducted in Turkey by Tiryaki et al[48] to evaluate the stool antigen test (HpSA) against

the urea breath test as the gold standard on a group of children with upper gastrointestinal complaints, a higher sensitivity and lower PPV of 86% and 84%, respectively, were found.

The molecular method applied in this study, which is PCR analysis of stool samples based on the *UreA* gene, revealed a sensitivity, specificity, PPV, and NPV of 72.2%, 100%, 92.9%, and 16.6%, respectively. This was in accordance with the results obtained by Vécsei et al[49] in a study conducted to detect *H. pylori* in stool samples of 143 children using commercially available real-time PCR based on *23S* rDNA. In another study using the specific primers for the *UreA* gene of *H. pylori* against histology-based tests, the results revealed higher sensitivity but the same specificity compared with the current study.[2]

Our values were higher than those obtained by Sen et al,[50] who conducted a study on a group of dyspeptic patients using the QIAamp DNA Stool Mini Kit for DNA extraction from stool and PCR using the *H. pylori UreA* gene (the same kit used in this study); the stool-PCR method and gold standard methods showed a statistical difference for the detection of *H. pylori* infection ($P < 0.0001$); sensitivity, specificity, PPV, and NPV were 65.22%, 75%, 93.75%, and 27.7%, respectively. Also, we recorded higher values than those obtained by Kabir[51] who compared different methods of stool extraction and the appropriate primers used. He concluded that DNA extraction from stool specimens using the QIAamp DNA Stool Mini Kit followed by PCR using the *UreA* gene recorded a sensitivity of 26% only, whereas specificity was not detected. Our higher figures could be attributed to the extreme care applied in this study while removing the inhibitory substances from stool samples during extraction.

We concluded that PCR for detection *of H. pylori* DNA in feces is a promising method for diagnosis, provided every effort is made to eliminate the effect of inhibitory molecules in stool samples, as the main limitation is the presence of inhibitors of the Taq polymerase used, which have been shown to be complex polysaccharides.[52]

CONCLUSIONS

Among the noninvasive methods for diagnosis of *H. pylori* infection, the 3 methods used in this study recorded promising results, including good sensitivity, which was the highest in the serum antibody test, whereas the stool-PCR test recorded excellent specificity. These results help us conclude that the invasive method can be replaced in the future. We recommend and encourage further large-scale studies that make the replacement of invasive techniques with noninvasive methods evidence-based.

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