The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89, Page 5129- 5135

Upper Endoscopy Biopsy Versus Helicobacter Pylori Antigen in Stool Test in Evaluation of Helicobacter Pylori Infection

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ABSTRACT

Background: Infection with the bacteria Helicobacter pylori is frequent in both young and old. Antibiotic susceptibility testing on an individual or community level, as well as data on antibiotic use and clinical outcomes, should inform the most effective local regimen for eradication. **Objective:** The aim of the current work was to evaluate the efficacy of commonly used antibiotics against Helicobacter pylori and to determine diagnostic stool antigen test accuracy performed after H. pylori medical treatment.

Patients and Methods: This prospective single arm cohort study included a total of 55 patients who tested positive for H. pylori, followed at Departments of Tropical Medicine and Pathology, Zagazig University Hospitals. This study was conducted between July 2020 and January 2021.

Results: H. pylori infection was more common in middle age group 32.6±8.58 and more in female (56.4%). The most frequent presentations were dyspepsia (63.6%), epigastric pain (23.6%) and abdominal fullness 12.7% of the cases. Gastritis 49.1%, duodenitis 32.7%, gastric ulcer 12.7% and duodenal ulcer 10.9%, are the common endoscopic diagnosis in H. pylori infection. After therapy, there was a moderate statistically significant agreement between biopsy and stool in diagnosis of H. pylori infection with sensitivity 90%, but specificity 73.3% and accuracy 76.4%.

Conclusion: It could be concluded that Helicobacter Pylori stool antigen test is highly sensitive with moderate specificity and accuracy for diagnosis of H. pylori infection post anti H. pylori therapy.

Keywords: Helicobacter, Antigen, Histopathology, Endoscopy; biopsy.

INTRODUCTION

Both toddlers and adults suffer from Helicobacter pylori infection at alarming rates [(1)]. Helicobacter pylori infects around 50% of the global population [(2)].

Helicobacter pylori are Gram-negative bacteria that do not produce spores [(3)]. Almost all cases of H. pylori infection are acquired, and at first, the vast majority of infected people show no signs of illness. Gastritis, gastric or duodenal ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer are only some of the gastrointestinal problems that can be caused by H. pylori infection throughout the course of a patient's lifetime, making this infection clinically significant. Infection with the bacterium Helicobacter pylori has been linked to health issues outside of the digestive tract, including malnutrition, iron deficiency anemia and stunted development [(4)].

Due to the rarity of natural eradication, infection typically persists for life in the absence of effective therapy [(1)].

The proper care of symptomatic H. pylori-infected individuals depends on prompt and accurate diagnosis. Several different H. pylori diagnostic tests based on the bacteria's morphological, immunological, genetic, or enzymatic properties have been established. Methods can be categorized as either non-invasive (stool antigen test, 13C-urea breath test as well as serology) or invasive (culture, urease test, histology) based on whether or not they necessitate endoscopy of the upper gastrointestinal tract and gastric biopsies. Each test has benefits, drawbacks, and restrictions depending on the clinical context and question being asked [(5)].

The cost of a stool antigen test is low, and while some patients may be hesitant to provide a fecal sample, doing so is typically painless. Enzyme immunoassays and immunological chromatography can be used to detect Helicobacter-specific antigen in stool samples [(5)].

Rapid in-office immuno-chromatographic stool antigen testing are as easy as a pregnancy test but less precise because they do not require a laboratory [(6)].

Monoclonal test kits for stool antigens are more sensitive than polyclonal testing. If a patient has diarrhea and their stools are loose and watery, the antigen concentration will be diluted, decreasing the sensitivity [(5)].

Rather than only testing for H. pylori infection to rule it out, it is suggested that patients with a family history of stomach cancer undergo diagnostic testing [(4)].

However, H. pylori testing may also be explored for people with iron deficiency anemia that has not responded to previous treatments. In both children and adults, H. pylori infection can be confirmed using a rapid urease test, a culture for H. pylori, and tissue staining using stomach tissues obtained during an endoscopy of the upper gastrointestinal tract. Histological testing of at least two tissue samples from the stomach antrum and body is required according to evidence-based criteria for H. pylori infection [(4)].

The optimum locally effective regimen for eradication should be based on either individual or community antibiotic susceptibility testing, or data regarding antibiotic use and clinical results [(7, 8)].

The effectiveness of eradication therapy should be monitored regularly (test for cure), preferably with noninvasive methods. For H pylori treatment to be

Received: 10/06/2022
Accepted: 17/08/2022

effective, it is suggested that at least 90% of cases be resolved to prevent the need for follow-up testing and antibiotics [9,10].

Testing to verify eradication with a urea breath test, fecal antigen test, or biopsy-based testing should be performed at least 4 weeks after terminating antibiotic treatment and after withholding PPI medicine for 1-2 weeks in patients who have been diagnosed with and treated for H. pylori infection [11].

The efficiency of current treatment options has decreased during the past decade due to the spread of antibiotic resistance. Testing for H. pylori infection after treatment has become increasingly necessary as a result [5].

Our study objectives were to evaluate the efficacy of commonly used antibiotics against Helicobacter pylori and to determine the accuracy of the diagnostic stool antigen test performed after H. pylori medical treatment.

PATIENT AND METHODS

This prospective single arm cohort study included a total of 55 patients who tested positive for H. pylori, followed at Departments of Tropical Medicine and Pathology, Zagazig University Hospitals. This study was conducted between July 2020 and January 2021.

Assuming the total number was 120 cases per year and the positive predictive value was 93% at 80% power and 95% CI the estimated sample size will be 55 cases in 6 months (Open Epi).

Inclusion criteria: Patients above 18years and below 60 years were included in the study. Patient presented with new-onset dyspeptic symptoms in patients with symptoms suggestive H. pylori infection (early satiety, bloating, and epigastric pain). Patients were diagnosed positive by H. Pylori stool antigen. Patients did not receive medical treatment for H. pylori.

Exclusion criteria: Patients aged below 18 years or above 60 years. Patients not fit for upper endoscopy. Patients had platelets count < 50,000/ cm^3 or prothrombin concentration < 60 %. Patients received medical treatment for H. pylori and Patients missed follow up.

The diagnosis of H. pylori infection depended on full history taking, clinical checkup, routine laboratory investigation: complete blood picture CBC, liver & kidney functions tests (LFTs, KFTs), coagulations profile (PT, PTT, and INR) and H. pylori antigen in stool test [12].

Specimen collection and technique: The obtained fecal samples were frozen at -70 degrees centigrade until analysis could be performed. ELISA technique was used for diagnosis of H. pylori antigen. Standard Diagnostics Inc.'s Yongin, Korea, SD H. pylori antigen ELISA kit was used.

Upper endoscopy examination with biopsy taking and histopathological examination:

In order to perform a safe and effective endoscopy under sedation, the drug propofol was employed. Posture: Examinee (patient): The starting position was lying on one's left side (lateral decubitus) (or if they experience difficulty lying in that manner, they can be examined in the supine position). Clinical Endoscopist/Inspector: Single-person EGD was chosen due to its efficiency. Different types of erosions and ulcers were identified during the endoscopic examination. If a patient had more than one illness, the most serious one was listed. Endoscopic visualization was used to guide biopsy specimen collection from the antrum, bulb of duodenum, and other sites for subsequent histological analysis. The biopsy samples were processed the next day, Hematoxylin and Eosin (H & E) and Giemsa staining after being fixed in 10% buffered formalin overnight, embedded in paraffin, and sectioned.

Follow up after H. pylori medical treatment: upper endoscopy examination with biopsy taking for histopathological examination and H. pylori stool antigen at least 4 weeks after the completion of medical therapy (14 days of 40 mg omeprazole, 500 mg clarithromycin and 1 gm amoxicillin) and after PPI withholding for 1–2 weeks.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The SPSS software, version 20 was used to analyze the data. Demographic data of the studied patients were presented as Mean ± Sd Range. McNemar test was used to evaluate statistical significance pre and post management. K: Crohon's Kappa test used to assess the significant of the validility analysis. To describe the qualitative data, we employed relative percentages and frequencies. The Mann-Whitney U test was applied to the data anyhow. The existence of a significant difference could be inferred from a P-value that was lower than 0.05.

RESULTS

This cohort research tracked 55 patients who tested positive for H. pylori., and H. pylori infection are dominant in middle age group and more common in female patients and normal range of the routine laboratory tests (**Table 1).**

Table (1): General Charters and routine laboratory tests among the studied patients

Variable		(n=55)	
Age: (years)	**Mean ±SD** **Range**	32.6±8.58 18-48	
Sex:		31 24	56.4 43.6
WBCs: ($x10^3/mm^3$)	**Mean ±SD**	5.54±1.05	
Hb: (g/dl)	**Mean ±SD**	12.39±1.39	
Platelets: ($x10^3/mm^3$)	**Mean ±SD**	286.53±61.87	
Urea: (mg/dl)	**Mean ±SD**	16.48±2.24	
Creatinine: (mg/dl)	**Mean ±SD**	0.75±0.16	
Bilirubin: (mg/dl)	**Mean ±SD**	0.84±0.14	
ALT: (IU/L)	**Mean ±SD**	31.44±7.73	
AST: (IU/L)	**Mean ±SD**	30.62±7.08	
ALP: (IU/L)	**Mean ±SD**	95.56±21.62	
Albumin: (g/dl)	**Mean ±SD**	4.24±0.45	
INR	**Mean ±SD**	1.04±0.04	

There were no significant relations between the routinely used laboratory investigations and H. pylori infections in the studied patients **(Table 2).**

Table (2): Relation between H pylori infection post treatment and Laboratory data of the studied patients:

Variable		-ve (n=45)	+ve (n=10)	t/MW	P
WBCs: ($x10^3/mm^3$)	Mean ± SD	5.61± 1.1	5.26± 1.38	0.44	0.66 NS
Hb: (g/dl)	Mean ± SD	12.33± 1.35	12.65± 1.63	0.66	0.51 NS
Platelets: ($x10^3/mm^3$)	Mean ± SD	294.04± 57.47	252.7± 62.55	1.97	0.06 NS
Urea: (mg/dl)	Mean ± SD	16.51± 3.22	16.32± 3.47	0.17	0.87 NS
Creatinine: (mg/dl)	Mean ± SD	0.73± 0.13	0.81±0.11	1.04	0.30 NS
Bilirubin: (mg/dl)	Mean ± SD	0.86±0.14	0.76±0.11	1.54	0.13 NS
ALT: (IU/L)	Mean ± SD	32.13±7.51	28.3±5.65	1.26	0.21 NS
AST: (IU/L)	Mean ± SD	30.8±7.07	29.8±7.2	0.40	0.69 NS
ALP: (IU/L)	Mean ± SD	94.4±21.1	100.8±18.42	0.30	0.77 NS
Albumin: (g/dl)	Mean ± SD	4.24±0.44	4.26±0.51	0.13	0.90 NS
INR	Mean ± SD	1.04±0.06	1.05±0.10	0.53	0.60 NS

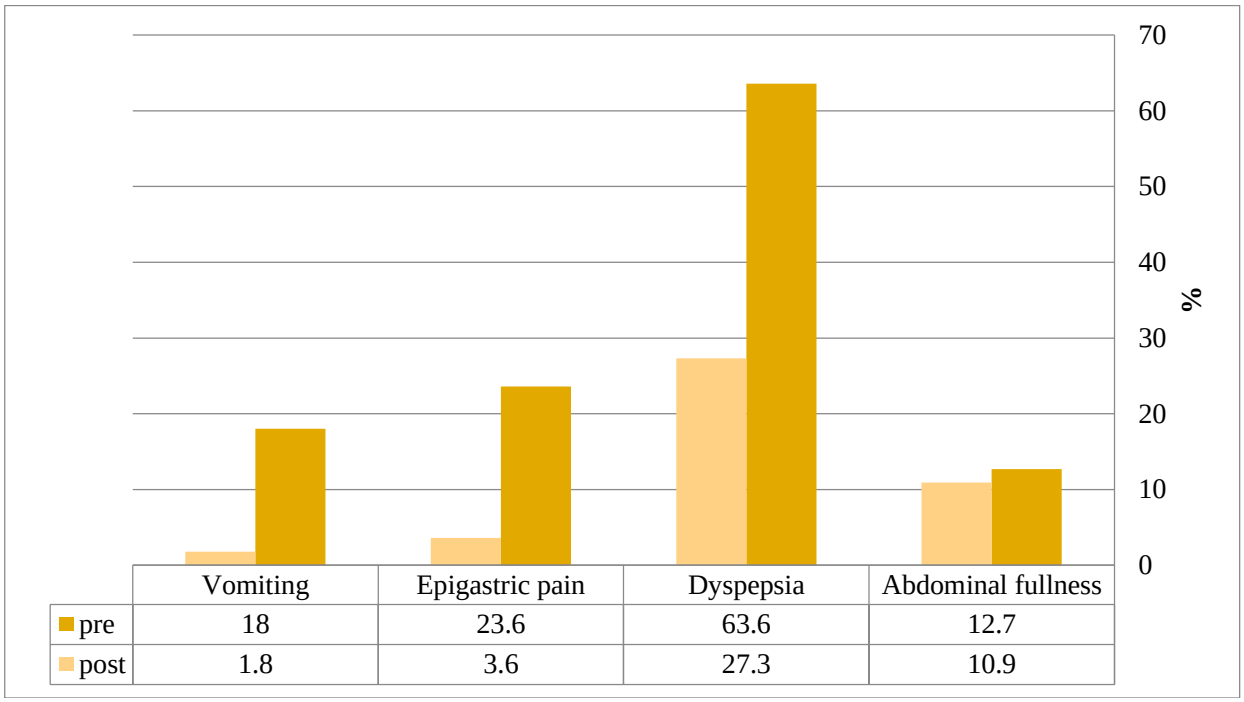
There was a moderate statistical significant agreement between biopsy and stool for diagnosing pylori infection with sensitivity 90%, but specificity 73.3% and accuracy 76.4% (Table 3).

Table (3): Validity of Stool antigen in diagnosis of H pylori after treatment in comparison to Upper endoscopy biopsy as a gold standard among the studied cases:

Stool	**Upper endoscopy Biopsy**		**Total**	**K**	**p**
	+ve	**-ve**			
+ve	9	12	21	**0.44**	**0.003***
-ve	1	33	34		
Total	10	45	55		
Validity	**Sensitivity:90% Specificit:73.3% PPV:42.9% NPV:97.1% Accuracy:76.4%**				

K:Crohon's Kappa test

The most frequent presentation findings among the studied cases pre therapy were dyspepsia (63.6%) followed by epigastric pain (23.6%), abdominal fullness presented in 12.7% and vomiting (18%) and persistence of the symptoms dyspepsia in 27.3%, abdominal fullness in 10.9% while decrease abdominal pain to 3.6% and vomiting 1.8% **(Figure 1)**. **Figure 3** shows endoscopic finding suggested H. pylori infection among the examined pre and post H. pylori therapy.



	Vomiting	Epigastric pain	Dyspepsia	Abdominal fullness
pre	18	23.6	63.6	12.7
post	1.8	3.6	27.3	10.9

Figure (1): Symptoms among the studied patients pre and post therapy.

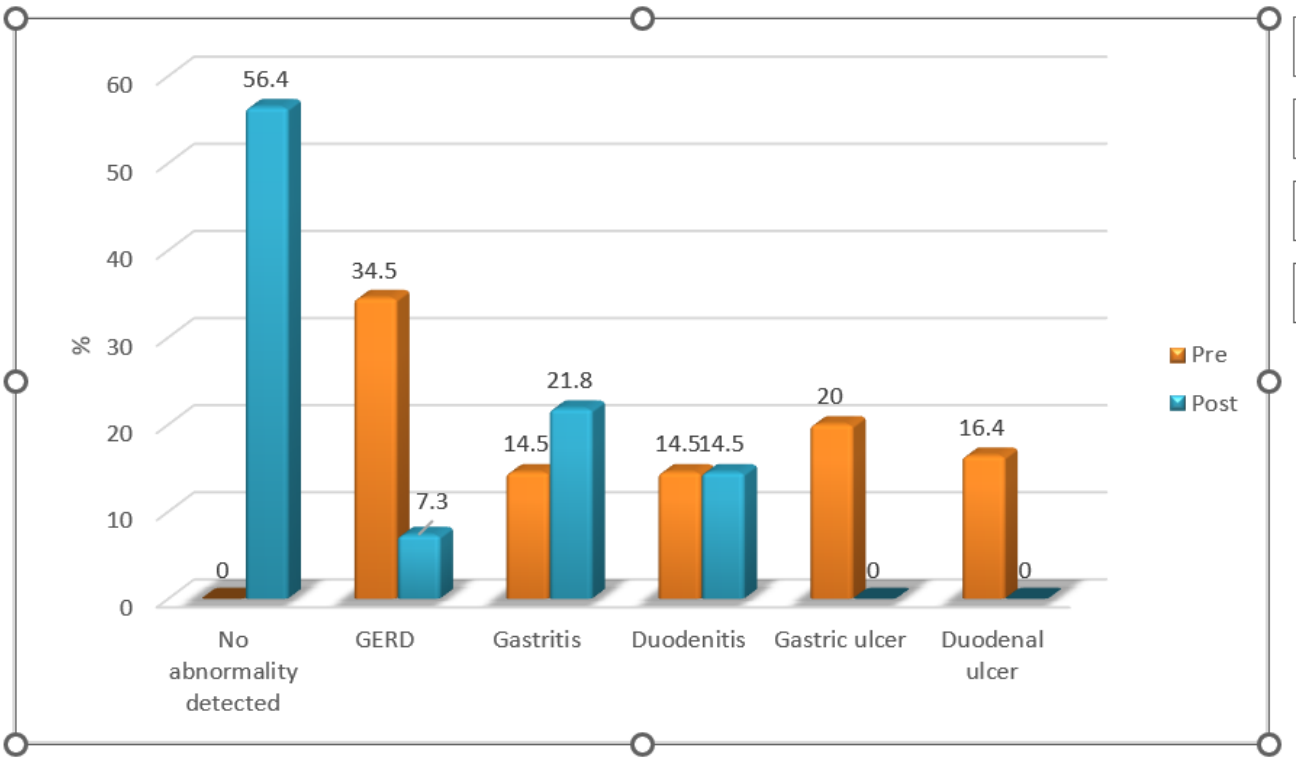


Figure (2): Endoscopy diagnosis among the studied group pre and post therapy.

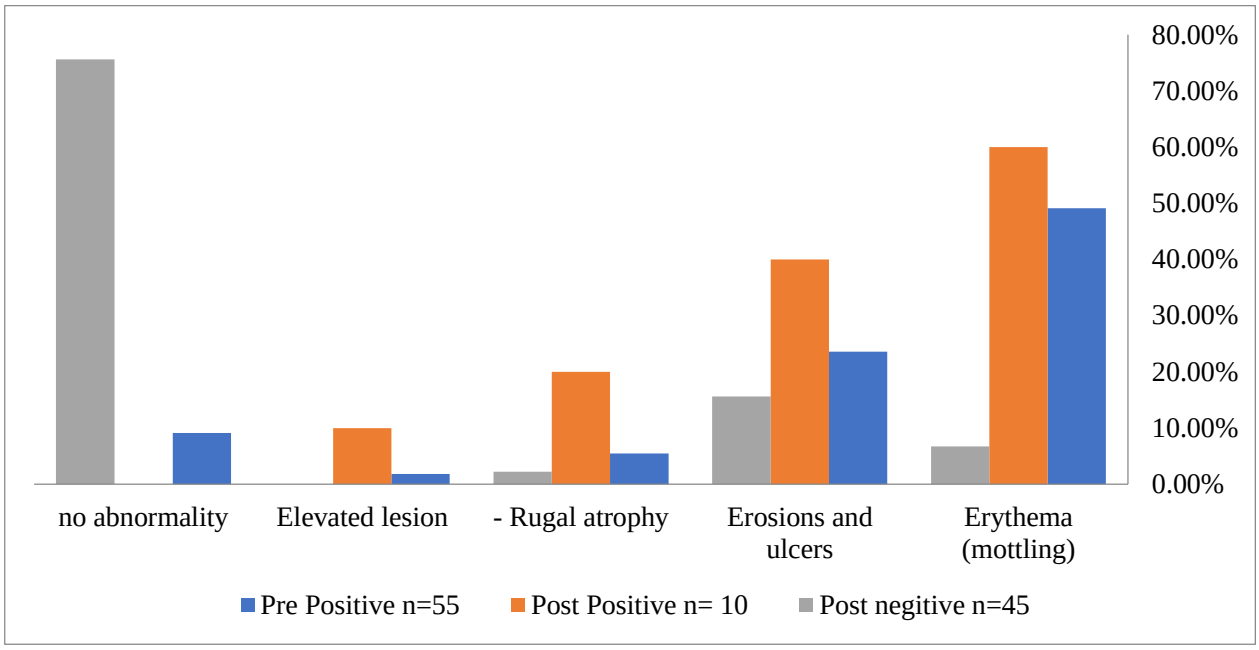


Figure (3): Endoscopic finding suggested H. pylori infection among the examined pre and post H. pylori therapy.

DISCUSSION

Gram-negative Helicobacter pylori bacteria live in the human stomach and cause many health problems. Most people in the world have H. pylori infection, making it the most common infectious condition [13].

H. pylori infection can be diagnosed with both invasive and non-invasive procedures. Cultures, histology, and urease tests are examples of intrusive techniques. Endoscopic biopsy samples from the esophagus, stomach, and duodenum are required for this testing. Stool antigen test (SAT), urea breath test, and serology are examples of noninvasive techniques [14].

A histological analysis of an endoscopic biopsy offers the highest sensitivity and specificity. However, the endoscopist's level of expertise, the quantity, location, and size of biopsies taken, as well as the staining and the pathologist's interpretation of the results, are all variables that might affect the accuracy of the diagnosis. While it is generally accepted that histopathology is the best method for detecting H. pylori, its application in clinical practice may be limited by factors such as its high cost, the lack of an adequate endoscope, and the lack of appropriately trained personnel [15]. This research set out to assess the effectiveness of routinely prescribed antibiotics against Helicobacter pylori and to evaluate the reliability of a diagnostic stool antigen test administered following medical therapy for H. pylori.

To elucidate this aim, 55 cases having H. pylori infection were included in the study.

In the present study, there was female predominance (56.4%), which near to the results in the study done by **Glover *et al.*** [16] who found that (56.9%) of their studied cases were females. But in the study was done by **Osman *et al.*** [17] there were 36 (61%) males and (39%) females. Also in the study had done by **Qadir *et al.*** [15] that composed of 110 patients, among them 80 were males (72.2%) and 30 females (27.27%) that difference may be attributable to the non-randomization in sample selection.

Adults are more likely to get an H. pylori infection. Subject ages in the original study ranged from 23 to 89 [14]. In this study, the mean age was 32.6±8.58 years ranging from 18 to 48 years which in agree with the results of **Sharbatdaran *et al.*** [18], they had found the mean age was 31.1±7.5 years while **Calik *et al.*** [14] reported the mean age was 45.02 ± 15.134 years. Also in another study by **Hussein *et al.*** [19] the age ranged from 18–70 years, which all were concerned in adult populations.

In the current study, The most frequent presentations findings among the studied cases pre therapy was dyspepsia (63.6%) followed by epigastric pain (23.6%), abdominal fullness presented in 12.7% and vomiting (18%) and persistence of the symptoms dyspepsia in 27.3%, abdominal fullness in 10.9% while decrease abdominal pain to 3.6% and vomiting 1.8% which was not following **Glover *et al.*** [16] who found that Chronic gastritis was the most common endoscopic findings in pre and posttreatment of their studied cases, also in the study was prepared by **Calik *et al.*** [14] via endoscopic biopsy, 86.8 percent were diagnosed with gastritis, and 13.1 percent were diagnosed with ulcer. **Hussein *et al.*** [19] discovered that 46.1% had antral gastritis, 24.3% had dyspepsia, and 16.5% had both stomach and duodenal ulcers.

As predicted, Peptic ulcers are largely attributed to the bacterium Helicobacter pylori, according to research [20]. According to **Talebi Bezmin Abadi** [21] physicians and microbiologists are keen to establish the best diagnostic approach because of the causal role H. pylori plays in duodenal ulcer and gastric cancer.

In our study, SAT was positive in all the studied cases pretreatment and 38.2% were positive post-treatment while in the study done by **Osman *et al.*** [17] Helicobacter pylori antigen test was positive in 22 patients and negative in 35.

In this study, there was a statistical significance decrease in frequency of infections detected by stool antigen among cases group post treatment compared to pre. Current recommendations state that the evaluation of eradication should be performed at least 4 weeks after the conclusion of eradication medication to reduce the likelihood of a false-positive result [22, 23, 24], and we followed these recommendations in in our study.

In regards to endoscopy findings, there was 9 cases had +ve findings in endoscopy and SAT and 33 cases had-ve findings by endoscopy and SAT which agree with **Calik *et al.*** [14] who stated that immunochromatographic assay results confirmed the endoscopic diagnosis in 85 of 91 (93.4%) patients with H. pylori-positive gastritis and 13 of 15 (86.6%) patients with H. pylori-positive ulcers.

Wang *et al.* [25] observed that SAT is the other major non-invasive approach, with sensitivity of 94% and specificity of 97%. The accuracy of stool antigen testing is compromised by a number of confounding factors. These include antibiotic use, proton pump inhibitor (PPI) use, N-acetyl cysteine use, bowel habits, and gastrointestinal hemorrhage.

In our study, After therapy, the stool antigen test was 76.4% as accurate as an Upper endoscopy biopsy in diagnosing H pylori, while **Cardos *et al.*** [26] stated that For diagnostic purposes, the SAT has an accuracy of over 90%. Regarding SAT method, a previous study by **Khalifehgholi *et al.*** [27] has stated that this test shows promise as a preliminary diagnostic tool and is beneficial for monitoring the development of H. pylori infection after therapy. H. pylori infection was found to have a 67% frequency using the SAT test. Similar findings were reported in a recent study by Egyptian researchers **Galal *et al.*** [28] who found a SAT method incidence rate of 64.6%. In contrast to this study,, **Al-Mashhadany** [29] employing the SAT approach, researchers in Iraq's Kurdish region found a significantly lower prevalence (11.3 percent). These distinctions may result from regional disparities in factors such as standard of living, literacy rate, diet, and sanitation...

In the current study, that there was a statistical significance decrease in frequency of infections detected by biopsy among cases group post treatment compared to pre that in agreement with **Glickman *et al.*** [30] **and Varbanova *et al.*** [31] who stated as Proton pump inhibitor (PPI) use is so common that it could cause gastritis to manifest atypically or cause localized differences in bacterial density. Using a specialized immune stain, digital pathology, or a special staining technique can increase the reliability of a histologic diagnosis of H. pylori infection.

In the present study, there was a moderate statistical significant agreement between biopsy and stool for diagnosing h pylori infection with sensitivity 90%, but specificity 73.3% which near to that was stated by **Dore and Pes** [32] who stated that the Endoscopic examinations have a sensitivity of 39% to 96% and a specificity of 83.6% to 100% This correlated nearly perfectly with the rates found by **Abd Rahim et al** [33]**.** The quality, location, size, and frequency of the biopsy as well as the applied dye variations have all been shown to affect the sensitivity of these tests, which can range from 50% to 95%[34]**.**

It is common knowledge that patients who have recently taken proton pump inhibitors (PPIs) or antibiotics can give misleading negative results due to decreased H. pylori colonization in the stomach, resulting in low H. pylori urease levels, or vice versa[32].

UBT had higher sensitivity, positive predictive /negative predictive values, and accuracy than SAT and the culture method in a comparison of three methods for diagnosing H. pylori, according to research by **Alzoubi *et al.*** [35] that was done at Jordan.

CONCLUSION

It could be concluded that Helicobacter pylori Stool Antigen Tests (HpSA) may be beneficial for the noninvasive diagnosis of H. pylori infection in adults due to its high sensitivity and moderate specificity when detecting H. pylori antigen, although when it is alone, it is not highly specific enough to detect H. pylori after treatment.

Financial support and sponsorship: Nil.
Conflict of interest: Nil.

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