Original Article

H. Pylori Fecal Antigen Detection versus Endoscopic Biopsy; Gold Standard in Dyspeptic Patients

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Abstract

Introduction: There are several invasive and noninvasive techniques used to diagnose H. pylori infection, each having its advantages and disadvantages. Invasive methods require biopsy samples from the stomach and duodenum and can be tested by various methods such as histology, Rapid urease test (RUT), microbiological culture, and Polymerase chain reaction (PCR), whereas noninvasive tests including stool antigen test, serology, and urea breath test.

Objective: To determine the diagnostic accuracy of the Helicobacter pylori fecal antigen test by taking endoscopic biopsy as the gold standard in dyspeptic patients.

Materials & Methods: Descriptive, cross-sectional study was conducted from 30th April 2019 to 30th October 2019 in Gastroenterology Unit, Holy Family Hospital, Rawalpindi. A total of 85 patients irrespective of gender aged 18–65 years with symptoms of dyspepsia (epigastric pain, early satiety, postprandial fullness, nausea, or retching) were included. Patients having gall bladder or pancreatic diseases, celiac disease, diabetes mellitus, thyroid disease, ischemic heart disease, chronic liver diseases, HIV, malignancy, alcoholism, pylori infection or treatment, drug PPI or H2 receptors, and pregnant were excluded. H. Pylori on fecal antigen detection and endoscopic biopsy was noted.

Results: Fecal antigen detection test found that 42 were True Positive and 04 were False Positive. Among 39, fecal antigen-negative patients, 04 (False Negative) had H. Pylori on endoscopic biopsy whereas 35 (True Negative) had no H. Pylori involvement on endoscopic biopsy (p=0.0001). Overall sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of H. Pylori Fecal antigen detection taking endoscopic biopsy as the gold standard in dyspeptic patients was 91.30%, 89.74%, 91.30%, 89.74%, and 90.59% respectively.

Conclusion: The study concluded that the diagnostic accuracy of Helicobacter pylori fecal antigen detection in dyspeptic patients is quite high.

Keywords: Helicobacter pylori infection, fecal antigen detection test, endoscopic biopsy.

Introduction

Helicobacter pylori is a motile flagellated microaerophilic spiral-shaped gram-negative bacteria, that involved up to 50% of the population suffering from chronic human bacterial infection.[1,2] The persistent chronic gastritis and gastritis involved gastric mucosal atrophy, and metaplasia, which later became gastric lymphoma and carcinoma. Helicobacter pylori infection was a carcinogen organism by WHO.[3] The noninvasive and invasive techniques were used to find Helicobacter pylori infection. Noninvasive tests were always preferable to test including serology/PCR, Urea breath test (UBT), and fecal antigen test. Many patients were refused to go for Invasive endoscopic biopsy samples from the stomach and duodenum, but it is considered a gold standard. Endoscopic biopsy samples and secretion are sent for Rapid urease test (RUT), polymerase chain reaction (PCR), histology, and microbiological culture. The latest guidelines of the European Helicobacter Study and American College of Gastroenterology recommended the eradication of Helicobacter pylori in patients who underwent gastric cancer resection.[4,5] However, no recommendations for a diagnostic test for H. Pylori gastric cancer patients.

H. pylori infection most commonly affects young age in the Pakistani population.[6] It is transmitted through three ways like person-to-person spread, oral-oral and fecal-oral.[7] Biopsy samples, observer-related variations, distribution of H. pylori, and type of stain used, have their limitations. These factors may give false results. The sensitivity and specificity were reported to be 90% for fecal antigen detection in the various studies.[8] Helicobacter pylori fecal antigen detection test can be considered a convenient noninvasive test as compared to upper GI endoscopic biopsy because it has shown the same results of sensitivity and specificity.[8-9] Helicobacter pylori infection is endemic in Pakistan enforcing an all-time threat as an an-emerging epidemic. Considering the fewer data published and knowledge on the test accuracy in dyspeptic patients in the local population, the objective was designed to determine the diagnostic accuracy of fecal antigen for Helicobacter pylori detection against the gold standard test used.

Objective: "To determine the diagnostic accuracy of H. Pylori fecal antigen detection taking endoscopic biopsy as gold standard in dyspeptic patients."

Operational Definitions:

- Dyspepsia: upper abdominal pain or discomfort, nausea or vomiting for 4 or more consecutive weeks and were assessed on history and examination.
- H Pylori Detection on Endoscopic biopsy: four quadrants of gastric antrum used to take biopsies and sent to hospital laboratory, then evaluated for histological examination (hematoxylin-eosin and modified Giemsa stain). Each biopsy was collected with 10% Formalin and paraffin-embedded solution. Each biopsy of 4mm thick histological sections was obtained, then examined under H & E stain and modified Giemsa stain. H pylori are usually detected in form of clusters in lumen of glands and on the surface epithelium.
- H Pylori Detection on H. pylori fecal antigen detection (HpfA): It is an immuno-assay that uses a monoclonal anti-H. pylori antibody. A diluent stool sample was spread into the test device after 5 min of incubation at room temperature, which shows the pink-red line in the reading Window next to the letter T indicating a positive result.
- Diagnostic Accuracy: By calculating sensitivity, specificity, positive predictive value, and negative predictive value which were mentioned below.

True positive: Presence of positive fecal antigen test and H pylori detected on biopsy.

True Negative: Presence of negative fecal antigen test and H pylori not detected on biopsy.

False positive: Presence of positive fecal antigen test and H pylori not detected on biopsy.

False negative: Presence of negative fecal antigen test and H pylori detected on biopsy.

Materials and Methods

A descriptive, cross-sectional study was conducted from 30th April 2019 to 30th October 2019 in Gastroenterology Unit, Holy Family Hospital, Rawalpindi. A total of 85 patients irrespective of gender aged 18–65 years with symptoms of dyspepsia (epigastric pain, early satiety, postprandial fullness, nausea, or retching) were included. Patients having gall bladder or pancreatic diseases, celiac disease, diabetes mellitus, thyroid disease, ischemic heart disease, chronic liver diseases, HIV, malignancy, alcoholism, pylori infection or treatment, drug PPI or H2 receptors, and pregnant were excluded. H. Pylori on fecal antigen detection and endoscopic biopsy was noted. The sample size was 85 at a 95% confidence level with expected sensitivity of 93% and specificity

of 80% with a prevalence of 51.3%.[11] The sampling technique was Non-probability and consecutive. Written informed consent was obtained from all participants before endoscopy and sample collection. Approval from the Institutional ethical committee was taken before the initiation of the study. According to the World Health Organization, the consent must be translated into a local language therefore it has been translated into Urdu for less education or common people. All enrolled patients were subjected to EGD for gastric mucosal biopsies (2 from antrum, 1 from incisura, 2 from body) for Helicobacter pylori detection and were sent to the hospital pathology laboratory and on the same day, their stool samples were collected. Stool specimens were tested using a commercially available kit i.e. SD BIO LINE H. Pylori Ag detection kit (Standard Diagnostic, Inc., Republic of Korea) according to the manufacturer's protocol. The test device and stool sample were settled at room temperature. Assay diluent was taken in the sample collection tube and with the help of a swab, a portion of feces about 500mg was taken and inserted into the sample collection tube containing assay diluent, 3 drops of this mixture (assay diluent and stool sample) was added to the sample well of the test device. Interpretation of test results was done within 10–15 min. The presence of two-color bands as test band (T) and control band (C) within the result window indicated a positive result. The presence of only the control band (C) within the result window indicated a negative result as per the kit instruction. All data were entered and analyzed using SPSS version 25.00. The frequency and percentages were calculated for qualitative variables like gender, and the presence of H. pylori infection. Quantitative data like age and duration of symptoms were presented as means or standard deviations. Diagnostic accuracy, specificity, Sensitivity, and positive and negative predictive values were calculated by Chi-square generating a 2x2 contingency table using formulas outlined in the operational definition. Values were considered significant having P-value <0.05 at a 95% confidence interval.

Results

The age range was from 18–65 years with a mean age of 43.02 ± 8.18 years. The majority of the patients 58 (68.24%) were 41-65 years of age. Of these 85 patients, 59 (69.41%) were males and 26 (30.59%) were females with a ratio of 2.3:1 (Figure I). The mean duration of the disease was 6.95 ± 1.84 months.

Fecal Antigen Detection found that 42 were True Positive and 04 were False Positive. Among 39, fecal antigen-negative patients, 04 (False Negative) had H. Pylori on endoscopic biopsy whereas 35 (True Negative) had no H. Pylori involvement on endoscopic biopsy (p=0.0001) as shown in Table4. Diagnostic accuracy of 90.59% of H. Pylori Fecal antigen detection taking endoscopic biopsy as the gold standard in dyspeptic Patients was 91.30% sensitivity, 89.74% specificity, 91.30% positive predictive value, and 89.74% negative predictive value respectively.

Table1: Distribution of patients according to the duration of disease

Duration of disease	*No. of Patients*	*%age*
≤6 months	34	40.0
>6 months	51	60.0
Total	85	100.0

Mean ± SD = 6.95 ± 1.84 months

Table 2: Diagnostic accuracy of H. Pylori Fecal Antigen Detection taking endoscopic biopsy as the gold standard in dyspeptic Patients

	Positive result on endoscopic biopsy	***Negative result on endoscopic biopsy***	***P-value***
Positive result on antigen detected	42 **(TP)***	04 **(FP)*****	**0.0001**
Negative result on antigen detected	04 **(FN)****	35 **(TN)******	

-TP=True positive **-FP=False positive *-FN=False negative ****-TN=True negative*

Sensitivity: 91.30%
Specificity: 89.74%
Positive Predictive Value (PPV): 91.30%
Negative Predictive Value (NPV): 89.74%
Diagnostic Accuracy: 90.59%

Table 3: Stratification of diagnostic accuracy with respect to age 18-40 years (n=27) and age 41-65 years (n=58)

Antigen detected	*Positive result on endoscopic biopsy 18-40 years (n=27)*	*Positive result on endoscopic biopsy 41-65 years (n=58)*	*Negative result on endoscopic biopsy 18-40 years (n=27)*	*Negative result on endoscopic biopsy 41-65 years (n=58)*	*P-Value*
Positive result	12 **(TP)**	30 **(TP)**	01 **(FP)**	03 **(FP)**	
Negative result	02 **(FN)**	02 **(FN)**	12 **(TN)**	23 **(TN)**	**0.001**

Table 4: Stratification of diagnostic accuracy with respect to female gender (n=26) and male gender (n=58).

ANTIGEN RESULTS	*Positive result on endoscopic biopsy FEMALE*	*Positive result on endoscopic biopsy MALE*	*Negative result on endoscopic biopsy FEMALE*	*Negative result on endoscopic biopsy MALE*	*P-Value*
Positive result	11 **(TP)**	31 **(TP)**	03 **(FP)**	01 **(FP)**	
Negative result	00 **(FN)**	04 **(FN)**	12 **(TN)**	23 **(TN)**	**0.001**

The Sensitivity is 100.0% in females and 88.57% in males. The Specificity in females was 80.0% as well as 95.83% in males. The Positive Predictive Value (PPV) was 78.57% in females than in males (PPV) 96.88%. The Negative Predictive Value (NPV) is 100.0% in females and (NPV) 85.19% in males. The Diagnostic Accuracy was 88.46% in females and 91.53% in males.

Discussion

Helicobacter pylori is a chronic infection involving up to 50% of the population from chronic human bacterial infection. Helicobacter pylori infection is an inescapable and challengeable management of peptic ulcer diseases and gastric adenocarcinoma. H. pylori first colonized and then produced a superficial persistent inflammation which led to gastroduodenal ulcer, mucosa-associated lymphoma, and cancer.[10-11] Clinicians and microbiologists are facing difficulty, but they are hopeful to find the best diagnostic approach.[12] Recently, there are many useful diagnostic methods used for H. pylori infection but only a few are recommendable having high sensitivity and specificity. H. pylori is perceived as a difficult organism to diagnose and treat, for which different samples are used e.g., saliva, gastric juice, gastroduodenal biopsy, urine, and stool with successful therapeutic practice globally.[12] Among them, non-invasive tests are always a preferable and convenient test for H. pylori such as antigen in the fecal sample, UBT (Urea Breath Test), and serology whereas invasive tests were difficult for patient and clinician to handle such as PCR (polymerase chain reaction), histology examination and culture which require endoscopic biopsy samples.[13] Each one has certain advantages and disadvantages.[13] The best management of H. pylori-related diseases depends on how to diagnose and eradicate H. pylori infection by following the patient vigilantly. The selection of drugs is highly dependent on the availability and feasibility in the hospital for poor patients.

The prevalence of 45% was reported by Khan et al[5] in Pakistan. The results of another study showed that (57%) 853 out of 1502 found seropositive for Helicobacter pylori. The prevalence of Helicobacter pylori infection was highest (63%) in the middle age (41–60 years) group as compared to (33%) in the teens and preteens (<20 years) group while 55% in the young age (20-40) and 60% in old age (>60 years). The study showed that 42 fecal Antigen Detection tests were True Positive compared to 04 were False Positive. Among 39 fecal antigen-negative patients, 04 (False Negative) had Helicobacter pylori on endoscopic biopsy whereas 35 (True Negative) had no Helicobacter pylori involvement on endoscopic biopsy (p=0.0001). Diagnostic accuracy of 90.59% of Helicobacter pylori fecal antigen detection taking endoscopic biopsy as the gold standard in dyspeptic Patients was 91.30% sensitivity, 89.74% specificity, 91.30% positive predictive value, and 89.74% negative predictive value respectively. Helicobacter pylori antigen in fecal samples has been detected by using polyclonal antibodies, the sensitivity of 88.8% and specificity of 94.5% have been found.[14] The Vaira et al[15] study showed that sensitivity of 94.1% and specificity of 91.8% in stool samples. The best benefit of the fecal antigen test is to eradicate Helicobacter pylori infection whereas if the concentration of antigen

becomes low, a false negativity test is found. Perri et al[16] study compared the performance of fecal antigen test versus Urea Breath Test in 458 dyspepsia patients, whereas an 8% discrepancy was found in these cases. The study found that the fecal antigen test was less accurate than the urea breath test. Nowadays, the latest generation of fecal antigen kits has been found by considering monoclonal antibodies despite polyclonal antibodies as compared to the urea breath accuracy test.

Gisbert et al[17] carried out a systematic review and a meta-analysis of diagnostic accuracy for the diagnosis of Helicobacter pylori infection of monoclonal SAT. Twenty-two studies having 2499 patients who evaluated the monoclonal SAT before eradication therapy showed that sensitivity was 94% whereas 97% specificity. In children, the study showed 100% sensitivity and 76.2% specificity to diagnose H. pylori infection by monoclonal SAT.[18] In another study, a sensitivity of 69% and specificity of 73% to diagnose H. pylori infection by the monoclonal SAT. The low sensitivity rate may be explained by the different cutoffs and qualitative variation of the SAT.[19] In a previous meta-analysis, H. pylori infection eradicated after therapy was 93% sensitivity and 96% specificity for monoclonal SAT.[20] In recent studies, monoclonal EIA-based SATs have specifically for eradication therapy of Helicobacter pylori with 91.6%-100% sensitivity as compared to 93.6%-98.4% specificity.[18] A meta-analysis of 45 studies having 5931 children to determine the performance of SATS was 92.1% sensitivity compared to 94.1% specificity. For the diagnostic test of Helicobacter pylori infection, Monoclonal SAT is the most important test.[21] The sensitivity of monoclonal SAT, polyclonal SAT, and one-step rapid monoclonal SAT were 96.2%, 94.7%, and 88.0% whereas the specificity of monoclonal SAT, polyclonal SAT, and one-step rapid monoclonal SAT were 93.0%, 88.1%, and 94.2% respectively.

Korkmaz et al. analyzed the diagnostic accuracy of different fecal antigen tests in dyspepsia patients relating monoclonal enzyme immunoassay tests and rapid immunochromatographic assays. The sensitivity was 48.9%–92.2% whereas specificity was 88.9%–94.4% compared to high variation. The study showed that monoclonal enzyme immunoassay tests are more specific compared to fast and easy use rapid immunochromatographic assays (ICA)-based tests but less reliable results.[22] Recently in forty-five studies, a meta-analysis carried out by Zhou and colleagues, 5931 children's assessed the Helicobacter pylori fecal antigen test which showed sensitivity was 92.1% whereas the specificity of 94.1%.[23] The fecal antigen tests have been able to differentiate infection from treated patients. The antigens were degraded in the intestine and the disintegration of epitopes leads to false negative results.[24]

Conclusion

The study showed that the diagnostic accuracy of Helicobacter pylori fecal antigen test in dyspepsia patients is quite high. The recommendation was fecal antigen detection test should be used as a primary test in the diagnosis of helicobacter pylori infection.

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