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Original Research Article

Evaluation of *Helicobacter pylori* Antigen Detection from Stool Samples for Diagnosis in Acid Peptic Disease Patients

Dr. Anuradha K[1*], Dr. Chandini MR[1], Dr. Bharathi Muniyappa[2], Dr. Dinesh HN[3], Dr.N.Vadiraj[4]
[1]Department of Microbiology, Mysore Medical College & Research Institute, Irwin Road, Next to Railway Staion, Mysuru, Karnataka, India
[2]Department of Pathology, Mysore Medical College & Research Institute, Irwin Road, Next to Railway Staion, Mysuru, Karnataka, India
[3] Department of Surgery, Mysore Medical College & Research Institute, Irwin Road, Next to Railway Staion, Mysuru, Karnataka, India
[4]Community Medicine, Mysore Medical College & Research Institute, Irwin Road, Next to Railway Staion, Mysuru, Karnataka, India
***Corresponding author:** Dr. Anuradha K
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Abstract

Helicobacter pylori infection is a widespread problem all over the world. It is the major cause of peptic ulcer disease and gastric carcinomas. Among the various methods available in clinical practice are histopathology, rapid urease test (RUT), cultureand PCR carried on gastric biopsy samples.Non-invasive diagnostics like stool antigen detection are available for rapid diagnosis and treatment follow up. The purpose of the study was to validate antigen detection of *H.pylori* from stool specimen and also to compare the test with that of rapid urease test and histopathology. This cross sectional case study was conducted on 260 subjects with symptoms of acid peptic disease who underwent endoscopic examination and not on non steroidal anti-inflammatory drugs or proton pump inhibitors. Gastric biopsy specimen and the stool samples were collected from patients with mucosal changes. Biopsy sample was subjected for Histopathological examination and Rapid urease test. Stool Antigen detection test was performed by immunochromatography method. Among the 260 study subjects stool antigen detection was positive in 184(70.7%) of the study population. *H.pylori* was detected by Rapid Urease test in 225(86.5%) of the patients while Histopathological examination identified *H.pylori* in 230(88.46%) by Haematoxylin and Eosin (H&E) staining and 235(90.38%) by Giemsa staining respectively. The sensitivity, Specificity, Positive predictive value and negative predictive value of Stool antigen detection when compared to Biopsy results were 78.29%, 100%, 100% and 32.89% respectively. The non invasive tests like stool antigen tests for *H.pylori* could be used as a routine diagnostic tool in the microbiology laboratory. The lower sensitivity of the stool antigen detection in comparison to histopathology and RUT is the drawback that has to be overcome.

Keywords: *Helicobacter pylori*, Rapid urease test, Stool Antigen test, Histopathology.

INTRODUCTION

Helicobacter pylori (H.pylori) is a Gram-negative flagellate bacterium that infects the stomach of more than half of the global population. Persistent *H. pylori* colonization of the human stomach is regarded as a risk factor for several diseases, including gastric adenocarcinoma, mucosa-associated lymphoid tissue lymphoma, and peptic ulceration. The WHO has classified *H. pylori* as a class I carcinogen, and a close association between infection and GC has been reported [1].

H. pylori-associated gastric cancer comprises about 5.5% of all cancers globally and accounts for 25% of all infection-associated cancers [2]. The prevalence of *H. pylori* shows large different geographical variations. Invarious developing countries, more than 80% of the population is *H.pylori* positive, even at young ages [3]. A variety of tests are available to diagnose *H. pylori* infection [4]. Diagnosis of *H. pylori* infection can be made with both invasive and noninvasive tests [5]. One of the common method is an invasive method based on endoscopy and gastric biopsies. Histopathology has been considered to be the gold standard test for detection of *H. pylori*infection [3]

The active use of PPI or antibiotics is known to cause false-negative results in all invasive tests. The rate of false-negative results is reported to be at least 30% [4]. Noninvasive tests should be employed for confirmation of eradication except in cases where repeat endoscopy is indicated. Stool antigen tests provide a noninvasive method for the detection of *H. pylori* [5]. Detection of *H. pylori* antigen in stool is an attractive noninvasive method that seems very suitable for clinical and epidemiologic studies [1].

The stool test detects the H pylori antigen present in stool in infected patients. The commercial stool antigen test (SAT) detects different antigens, the

results change from one test to another. Furthermore, because of the marked genetic variability of *H. pylori*, the accuracy of the same test may vary according to population. For this reason, the Maastricht consensus recommends local validation before using a given SAT [6]. SAT may use polyclonal or monoclonal antibodies [4]. Meta-analyses have shown that SAT using polyclonal antibodies are consistently inferior to those using monoclonal antibodies [7].

Traditional diagnosis is made using a combination of tests, both invasive and noninvasive. The choice of tests usually depends on clinical circumstances, the likelihood ratio of positive and negative tests, the cost effectiveness of the testing strategy and of the availability of the tests [8].

The present study was undertaken to validate antigen detection of *H.pylori* from stool specimen by Immunochromatography test with that of rapid urease test and histopathology from biopsy specimens.

MATERIALS & METHODS

This was a cross sectional case study. The subjects included were the patients with symptoms of acid peptic disease who underwent endoscopic examination and not on non steroidal anti-inflammatory drugs or proton pump inhibitors. As per the statistical calculation 260 subjects were included in the present study after taking informed consent.

Gastric biopsy specimen was collected from the area of mucosal changes and all the subjects were requested to submit the stool sample.

Biopsy sample was subjected for-

- Histopathology examination by Hematoxylin and Eosin staining and Giemsa staining
- Rapid Urease test- biopsy sample was inoculated into the urease medium and looked for change in colour.

Stool antigen detection was performed using Immunochromatographic test (ICT) as per the manufacturer's instruction. This qualitative test is read after 20 min of incubation at room temperature. Results were reported according to the following criteria: the presence of 2 lines (test and control) is considered as positive, the presence of only 1 line (control line) is considered negative, the presence of only 1 line (test line) or the absence of the control line is considered as an invalid test.

RESULTS

A total of 260 patients were enrolled in the present study. Among the study population 182(70%) were males and 78(30%) were females. The age and gender distribution of the study population is shown in Table-1.

Table-1: Age and gender distribution of the patients

Age Group	Distribution of study population	Male	Female
20-29	22	16	6
30-39	76	47	29
40-49	85	58	22
50-59	51	39	12
60-69	22	18	04
70-70	04	04	-

In the study population Rapid Urease test was positive in 225(86.5%), Stool antigen detection was positive in 184(70.7%). Histopathological examination detected *H.pylori*by Haematoxylin and Eosin (H&E) staining in 230(88.46%) and 235(90.38%) by Giemsa staining respectively.

In the histopathological examination presence of typical bacteria along with the inflammatory reaction in the tissue slides was studied for *H. pylori* infection. Giemsa staining was used for *H. pylori* detection. The Haematoxylin and Eosin stain helped in evaluation of severity of inflammation along with detection of the bacteria. The Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and diagnostic accuracy of H & E in comparison to Giemsa in the present study was 95.74%, 100%, 100%, 91.42% and 96.15% respectively.

The sensitivity, Specificity, Positive predictive value and negative predictive value of Rapid urease test when compared to Biopsy results were 95.7%, 92.59%, 99.11% and 71.42% respectively. The sensitivity, Specificity, Positive predictive value and negative predictive value of Stool antigen detection when compared to Biopsy results were 78.29%,100%, 100% and 32.89% respectively (Table-2).

Table-2: Statistical analysis of the diagnostic tests

Diagnostic test	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy*
Rapid urease test	95.7%	92.59%	99.11%	71.42%	95.38%
Stool Antigen detection	78.29%	100%	100%	32.89%	80.38%

*diagnostic accuracy in comparison with histopathological results

Using Histopathological results as the gold standard we obtained good diagnostic accuracy with the RUT (95.38%). The stool antigen detection test reported false negative tests and the diagnostic accuracy proved to be 80.38% when compared to biopsy results.

DISCUSSION

The diagnosis of *H.pylori* associated acid peptic disease is done through many tests. We studied gastric biopsy from 260 subjects by means of histopathological study, Rapid urease test and non invasive stool antigen detection by immunochromatography test. The majority of the study subjects were in the age group of 40-49 years (32%). Males constituted 70% of the study population.

The *H.pylori* was demonstrated by histopathological study, 235(90.38%) by giemsa stain and 230(88.46%) by Hematoxylin and eosin staining method. Choi *et al*., evaluated different diagnostic methods in the specific setting of peptic ulcer, concluding that histology was the most accurate test, compared with culture, serology and RUT [9]. Ramirez-Lazaro *et al.,* found that IHC and real-time PCR methods might improve the sensitivity of biopsy-based diagnosis in this specific setting of peptic ulcer bleeding episodes [10].

Histology has been the mainstay of the invasive diagnosis of H pylori infection. Its global reliability remains high in recent studies, with sensitivity and specificity rates higher than 95% [11]. Giemsa is generally preferred to hematoxylin-eosin. Giemsa stain is routinely performed at most centers and is cheap and highly reliable for the diagnosis of H pylori infection. By contrast, it has been suggested that hematoxylin-eosin alone has a lower sensitivity for diagnosing *H pylori* infection [12-15].

In the present study the rapid urease test showed a sensitivity and specificity of 95.7% and 92.59% respectively. Commercial RUTs have a sensitivity of 80% to 95% and specificity higher than 95%to 100% [16, 17]. The number of bacteria present in the biopsy is the main cause of the reduction in sensitivity. It is estimated that densities lower than $10^4$to 10^5 organisms may result in false-negative tests [18]. Overall, the test is cheap and rapid and provides adequate screening.

The sensitivity and specificity of the stool antigen detection in the present study was 78.29% and 100% respectively. A major problem with the use of SAT is that many commercial tests are available but have never been validated; in addition, in some areas, sensitivity and specificity ofeven the best monoclonal ELISA SAT barely reaches 90% [19, 20]. Besides being non-invasive, the advantages of using this method include the unneeded requirement of expensive equipment and medical personnel, and the collection of the sample at home without a visit to the hospital. A meta-analysis revealed that the global sensitivity and specificity of stool antigen tests are 94% (95%CI: 93-95) and 97% (95%CI: 96-98), respectively [21].

In a Japanese study, the *H.pylori* stool antigen test had a reported sensitivity of 93.9% and specificity of 95.7%, compared to a diagnosis of infection based on histological examination [22]. However, Blanco *et al.,* have observed that another stool antigen test showed a low sensitivity (75%-79%), in patients with *H. pylori* infection who were tested after erradication therapy [23]. In the present study the stool antigen test results had a low but acceptable correlation with the histological examination.

CONCLUSION

Recent developments in both biopsyand non-biopsy based diagnostic methods for *H. pylori* infection have further contributed to improving current clinical approach and management of *H. pylori*-associated diseases.In conclusion, the RUT for the detection of *H. pylori* in this study, was found to correlate with histological examination as a gold standard. In addition, there was a little conflicting result on Stool antigen detection when compared to histological examination. The stool antigen test is a rapid, simple, and noninvasive test with acceptable results. Therefore, histopathological examination as a gold standard and the RUT along with stool antigen detection may be the preferred methods to use for the precise detection of *H. pylori*.The specific contribution of each method to the evolving strategies and algorithms for evaluation and management of *H. pylori* infection will remain of paramount relevance.

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