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DIAGNOSTIC ACCURACY OF STOOL ANTIGEN TEST FOR HELICOBACTER PYLORI DIAGNOSIS AT GIMS HOSPITAL; GAMBAT

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ABSTRACT

Objective: To assess the diagnostic value of the stool antigen test for Helicobacter pylori (H pylori) infection compared to gastric biopsy histopathology (gold standard).

Methods: It was a cross-sectional validation study done in the outpatient and inpatient Department of Medicine at GIMS Hospital, Gambat for duration of six months. The patients were consecutively sampled as non-probability sampling of 226 patients aged between 18–60 years who had peptic ulcer disease as a suspected diagnosis. Stool samples were analyzed for Helicobacter pylori antigen using an immunochromatographic assay and upper gastrointestinal endoscopy and gastric mucosal biopsy were performed. Histopathology was used as the reference standard. These SPVs, PPVs, NPVs and diagnostic accuracy were computed in SPSS 26.0.

Results: The mean age was 41.7 ± 11.2 years, and 121 (53.5%) participants were male. Gastric biopsy was positive for Helicobacter pylori in 124 (54.9%) patients and the stool antigen test in 116 (51.3%) patients. The numbers of true-positive, of true-negative, of false-positive and of false-negative results were 94, 80, 22, and 30, respectively. The sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy were 75.8%, 78.4%, 81.0%, 72.7% and 77.0%, respectively.

Conclusion: Stool antigen test had moderate diagnostic performance and could be used as a good non-invasive test to detect Helicobacter pylori infection; however, negative or discordant results should be interpreted with caution when final diagnosis is needed in clinical practice.

KEYWORDS: Helicobacter pylori; stool antigen test; gastric biopsy; diagnostic accuracy; sensitivity; specificity.

INTRODUCTION

Helicobacter pylori is a spiral-shaped, gram-negative bacterium that colonizes the gastric mucosa and is an important cause of chronic gastritis, peptic ulcer disease, gastric lymphoma, and gastric carcinoma. Early diagnosis of the active infection is crucial for the proper treatment and prevention of complications. The diagnostic tests used for the detection of H. pylori can be divided into biopsy-based and non-biopsy-based and the choice of the tests depends on the clinical context, patient's characteristics and availability of tests [1].

New diagnostic technologies have made a number of tests available such as histology, rapid urease testing, culture, urea breath testing, serology and stool antigen testing. Non-invasive tests decrease patient discomfort and risk of the procedure, and allow evaluation of active infection [2]. Laboratory diagnosis is still problematic, due to the potential for variable test sensitivity depending on the load of bacteria, the way bacteria are handled in the laboratory, exposure to antibiotics, proton pump inhibitors, and prevalence of the disease [3].

Stool antigen test is becoming more popular for the initial diagnosis and confirmation of eradication of H. pylori antigens in stool. LFIAs are rapid, and while they can be very useful in resource-limited environments, their performance can vary depending on the antibody system and assay design [4]. Newer assays that use enzymes in the stool antigen may have better analytical performance and may be more sensitive in detecting active infection [5]. Stool antigen testing has also been demonstrated to have acceptable diagnostic performance in population studies when compared with reference tests [6].

In fact, the accuracy of diagnosis varies between commercially available assays. Sensitivity and specificity have been reported to vary by population and health facility [7]. Comparative assessment of rapid stool antigen assays has revealed that the various assays might not accurately identify infected and non-infected patients [8]. There have been some similar discrepancies noted when comparing newer stool antigen platforms and the need for platform-specific local validation before rolling out the platform routinely. Antigen shedding could be affected by proton pump inhibitor therapy because it can lead to decreased bacterial activity and to false-negative results [10].

Rationale: This study was performed because there was a lack of local evidence available on the diagnostic accuracy of stool antigen testing at GIMS Hospital, Gambat. It was felt important to evaluate its sensitivity, specificity and diagnostic performance to see if this non-invasive test would have enough sensitivity to be used reliably for H. pylori diagnosis in the local population.

METHODS

The study was of cross-sectional validation type carried out in the Outpatient and In-patient Department of Medicine at GIMS Hospital, Gambat. The study was undertaken over three months from 4 August 2025 until 3 November 2025. The study was approved by the Gambat Medical College, Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences, Gambat, Ethical Review Committee Approval No. **PAQSJIMS/779/25-RC**, dated 16 July 2025.

Sample size was determined by using a sensitivity and specificity calculator using the expected prevalence of Helicobacter pylori infection of 50.89%, the expected sensitivity of the stool antigen test of 66.1%, the expected specificity of 82.5%, and the margin of error of 8%. Non-probability consecutive sampling was used and a total of 226 patients were included. Patients, both male and female, aged 18–60 years with clinical suspicion of peptic ulcer disease (PUD) who were scheduled for upper gastrointestinal endoscopy (UGE) were recruited for the study. Peptic ulcer disease was clinically suspected when patients presented with upper abdominal pain with a score of ≥4 on visual analogue scale, and dyspepsia for more than six weeks, with or without weight loss of ≥5 kg in one month, hematemesis, or melena. Patients were excluded if they had taken antibiotics during the last 4 weeks, had a history of H. pylori eradication therapy, had chronic kidney disease, decompensated chronic liver disease, or were pregnant.

Demographic and clinical details such as age, sex, residence and duration of symptoms were collected in a predesigned proforma after taking written informed consent. Stool samples were collected from all the participants and tested for H. pylori antigen by immunochromatographic method. The stool antigen test was positive if two separate pink or red bands were present, one in the test area and one in the control area. Then each participant had an upper gastrointestinal endoscopy in which they had a biopsy of their stomach lining at the edge of the peptic ulcer. Histopathological examination of the gastric biopsy was used as the gold standard. The diagnosis of H. pylori infection was established by the presence of organisms on the gastric mucosal surface epithelium, irrespective of the presence or absence of inflammatory infiltrates, lymphoid follicles, or glandular atrophy. All observations were made by the principal investigator and confidentiality of the participants and the data was ensured.

SPSS 26.0 software was used to enter and analyse the data. Shapiro–Wilk test was used to evaluate the distribution of continuous variables. Age and duration of symptoms were reported as mean ± standard deviation or median and interquartile range, as appropriate, and sex and residence were reported as frequencies and percentages. A 2 × 2 contingency table was created according to stool antigen test and gastric biopsy histopathology results. The sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy were determined. Stratified analyses were done by age, sex, and residential status, and the diagnostic performance measures within these strata were re-estimated.

RESULTS

A total of 226 patients suspected of having peptic ulcer disease were included in the study. The mean age was 41.7 ± 11.2 years, with 121 (53.5%) males and 105 (46.5%) females. Most patients were urban residents (58.4%). The median duration of symptoms was 10 weeks (IQR: 8–16 weeks). Gastric biopsy histopathology confirmed Helicobacter pylori infection in 124 (54.9%) patients, whereas the stool antigen test was positive in 116 (51.3%) patients (**Table 1**).

Comparison of stool antigen testing with gastric biopsy histopathology showed 94 true-positive, 80 true-negative, 22 false-positive, and 30 false-negative results. The stool antigen test demonstrated a sensitivity of 75.8%, specificity of 78.4%, positive predictive value of 81.0%, negative predictive value of 72.7%, and overall diagnostic accuracy of 77.0% (**Table 2**).

On subgroup analysis, diagnostic accuracy was relatively higher among patients aged 18–40 years (79.5%) than those aged 41–60 years (74.6%). Diagnostic performance was comparable between males and females, with accuracies of 76.9% and 77.1%, respectively. Urban participants demonstrated slightly higher sensitivity, specificity, and overall diagnostic accuracy than rural participants (**Table 3**).

Table 1. Baseline demographic and clinical characteristics of study participants (n = 226)

Variable	Value
Age, years, mean ± SD	41.7 ± 11.2
Age group	
18–40 years	112 (49.6%)
41–60 years	114 (50.4%)
Sex	
Male	121 (53.5%)
Female	105 (46.5%)
Residence	
Urban	132 (58.4%)
Rural	94 (41.6%)
Duration of symptoms, weeks, median (IQR)	10 (8–16)
Gastric biopsy positive for H. pylori	124 (54.9%)
Gastric biopsy negative for H. pylori	102 (45.1%)
Stool antigen test positive	116 (51.3%)
Stool antigen test negative	110 (48.7%)

Values are presented as n (%), mean ± standard deviation, or median (interquartile range), as appropriate.

Table 2. Diagnostic performance of stool antigen test compared with gastric biopsy histopathology (n = 226)

Stool antigen test	Gastric biopsy positive	Gastric biopsy negative	Total
Positive	94 (True positive)	22 (False positive)	116
Negative	30 (False negative)	80 (True negative)	110
Total	124	102	226
Diagnostic parameter	Value, %	95% CI	
Sensitivity	75.8	67.6–82.5	
Specificity	78.4	69.5–85.3	
Positive predictive value	81.0	73.0–87.1	
Negative predictive value	72.7	63.7–80.2	
Overall diagnostic accuracy	77.0	71.1–82.0	

Gastric biopsy histopathology was considered the gold standard.

Table 3. Subgroup analysis of diagnostic performance of stool antigen test

Subgroup	n	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
Age group						
18–40 years	112	78.3	80.8	82.5	76.4	79.5
41–60 years	114	73.4	76.0	79.7	69.1	74.6
Sex						
Male	121	76.5	77.4	81.3	71.9	76.9
Female	105	75.0	79.6	80.8	73.6	77.1
Residence						
Urban	132	78.1	79.7	82.6	74.6	78.8
Rural	94	72.5	76.7	78.7	70.2	74.5

PPV: positive predictive value; NPV: negative predictive value

DISCUSSION

Gastric biopsy in the present study yielded a positive result for H. pylori infection in 54.9% while stool antigen test was positive in 51.3% of the participants. The stool antigen assay had a sensitivity of 75.8%, specificity of 78.4%, positive predictive value (PPV) of 81.0%, negative predictive value (NPV) of 72.7%, and diagnostic accuracy of 77.0%. These results show moderate diagnostic accuracy, suggesting that stool antigen testing may be a valuable non-biopsy-based of investigation, but should not be used to replace biopsy-based confirmation in all clinical scenarios. A systematic review and meta-analysis of stool antigen tests in the elderly patient also demonstrated that stool antigen tests are a valuable non-biopsy-based method, but also showed the heterogeneity of the studies available [11].

Diagnostic literature highlights the importance of considering assay characteristics, patient factors, specimen quality, bacterial load, medication exposure and the reference standard in determining the performance of H. pylori tests. Therefore, reviews of available diagnostic methods state that the results of stool antigen tests should be interpreted based on the clinical context and the technical features of the test [12]. Kubo et al found very high

specificity in all non-infected groups and high sensitivity (97.3%) in currently infected individuals when compared to our results using a newly developed stool antigen assay [13]. This discrepancy may be due to differences in assay technology and/or population variation and/or differences in the definition of the reference.

The significance of assay methodology is further highlighted by the findings of Imagawa et al., who showed very high levels of agreement when the bioluminescent enzyme immunoassay was used, with different stool collection containers [14]. However, in Charach et al.'s institutional comparison, the 13C-urea breath test outperformed stool antigen testing, highlighting key limitations of the stool test (its convenience) [15]. Another recent meta-analysis in adults also showed that conventional stool antigen assays are more accurate than rapid stool antigen tests and that there are differences in performance depending on the type of antibody tested and the method of assessment used [16].

The present results were inferior to some of the recent studies employing monoclonal antibody-based techniques. Song et al. found good performance of monoclonal stool antigen testing even among patients with acute non-variceal upper gastrointestinal bleeding [17]. Kang et al. also indicated that the sensitivity and specificity of stool antigen detection are >90%, especially with the use of the monoclonal antibody-based assay; however, the specimen handling and bacterial load may influence the results [18]. Fang et al. then showed that there was a high specificity for the monoclonal stool antigen test and strong diagnostic consistency in patients with chronic atrophic gastritis [19]. Raso et al. found stool antigen to be sensitive in 94% of pediatric patients, further supporting the utility of optimized stool antigen assays in clinical populations [20].

STRENGTHS AND LIMITATIONS

A strength was that stool antigen testing and gastric biopsy histopathology were compared in the same participants. Sensitivity, specificity, predictive values and overall accuracy were also assessed. The limitations were that it was a single-center study, non-probability sampling, and used a single stool antigen testing method. The exposure time to medication, the handling of specimens, and the level of bacteria may also have impacted performance.

RECOMMENDATIONS

Additional multicentre studies of larger size should be used to test various monoclonal and laboratory-based stool antigen assays and to directly compare them to histopathology and urea breath testing. The collection of specimens should be standardized and proton pump inhibitor and antibiotic exposure should be controlled.

CONCLUSION

The sensitivity, specificity and overall diagnostic accuracy of stool antigen testing for the diagnosis of H. pylori were moderate. It was definitely a convenient and non-invasive diagnostic possibility, but negative or clinically discordant responses to this test should be viewed with caution, especially when definitive diagnosis is needed.

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