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Comparative Assessment of *Helicobacter pylori* stool Ag and serum Anti-bodies Tests with Urea Breath Test among Symptomatic Patients at a Tertiary Hospital in Kabul, Afghanistan

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ABSTRACT

Background: Helicobacter pylori infection is a major cause of gastrointestinal disorders. Accurate diagnosis is essential for proper management, particularly in resource-limited settings. We compared the diagnostic performance of stool antigen (Ag) and serum antibody (Ab) tests with the Urea Breath Test (UBT), the gold standard for detecting H. pylori infection, among symptomatic patients at a tertiary hospital in Kabul, Afghanistan, in 2023.

Methods: This case series study included dyspeptic patients attending the outpatient department of Cure Hospital, Kabul, Afghanistan, who had not used proton pump inhibitors in the previous two weeks or antibiotics in the past four weeks. All patients underwent stool antigen testing, serum antibody testing, and UBT. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) were calculated to assess diagnostic accuracy.

Results: Of the 66 patients enrolled (mean age = 36.4 ± 13.2 years; 68.2% were female), abdominal pain (93%) was the most common symptom. The stool antigen test showed high sensitivity (96%) and specificity (97.5%), while the serum antibody test exhibited lower sensitivity (73%) and specificity (67.5%). Sex-stratified analysis showed consistently high accuracy across for the stool antigen test across both sexes, while the performance of the antibody test varied by sex.

Conclusion: The stool antigen test shows high sensitivity and specificity and performs better than the serum antibody test for diagnosing H. pylori infection among symptomatic patients. It can be considered a reliable alternative to UBT in resource-limited settings.

Keywords: *Helicobacter pylori*, Stool antigen, Antibody test, Urea breath test, Diagnosis, Afghanistan

Introduction

Helicobacter pylori is recognized as the primary cause of gastritis and peptic ulcer disease, and it is also linked to the development of gastric cancer (1). The prevalence of *H. pylori* infection varies globally, with developed nations reporting less than 50%, while this prevalence ranges between 85% to 95% in developing countries (2-5). Socioeconomic status, sanitation, and geographic location are among the factors influencing this inequality (6, 7). Inadequate hygiene, overcrowded living conditions, and limited access to clean water have been consistently linked to increased *H. pylori* transmission (8). Persistent *H. pylori* infection can lead to various gastrointestinal diseases, including gastritis, ulcers, and gastric lymphoma (9). Furthermore, its association with extra-gastric conditions such as immune thrombocytopenia and Alzheimer's disease, highlights its broad impact on health (10-14). The WHO recognized *H. pylori* as a significant contributor to gastric cancer, emphasizing the need for effective management and control strategies (15).

In Afghanistan, the public health landscape faces profound challenges, particularly in controlling infectious diseases, despite two decades of improvements and achievements in the health sector. There are still fundamental challenges in controlling infectious diseases such as tuberculosis, malaria, HIV, measles, and cholera, which claim hundreds of lives each year. Infections such as *H. pylori* have become a growing concern in Afghanistan, with prevalence of approximately 76% which emphasizes the urgent need for accessible and reliable diagnostic tools in both urban and rural settings (16).

Considering the rising global prevalence of *H. pylori* infection, early detection and management are critical to mitigate its consequences, particularly in the Low-and Middle-Income Countries (LMICs). Diagnostic methods for *H. pylori* can be categorized into invasive and non-invasive techniques. Non-invasive methods are typically employed as initial diagnostic tests and include serological tests (17), the 13C-urea breath test (UBT), and stool antigen tests that detect *H. pylori* antigens in stool samples. While serological tests are relatively inexpensive and widely used for screening infections, their accuracy remains a concern, making them inappropriate for monitoring treatment success (18).

In contrast, the UBT is considered the gold standard for non-invasive diagnosis, with reported sensitivity and specificity rates in the range of 90-95% (19). Stool antigen tests, particularly those using enzyme immunoassays like ELISA, have also shown promising diagnostic accuracy and are increasingly adopted in primary care settings (17, 19, 20). However, the available spectrum of these diagnostic approaches for *H. pylori* infection varies in affordability among the general public, raising questions about the accuracy, sensitivity, and specificity of each test compared to standard methods. We aimed to evaluate and compare the diagnostic performance of Stool Antigen (Ag) and Serum antibody tests (Ab) against the Urea Breath Test (UBT), considered the gold standard for detecting *H. pylori* infection among symptomatic patients. By focusing on these specific diagnostic tools in an Afghan context, this research seeks to provide insights that can inform local healthcare practices and improve patient outcomes. In summary, while prior studies have highlighted the importance of accurate *H. pylori* diagnosis globally, this study uniquely addresses the pressing need for reliable diagnostic approaches within Afghanistan's healthcare framework, offering valuable data may differ from findings in other regions.

Methods

Study design and study setting

This was a case-series diagnostic accuracy study conducted between September 2023 and December 2023 at the Family Medicine Department of Cure Hospital, Kabul, Afghanistan. We aimed to evaluate and compare the diagnostic performance of the stool antigen and serum antibody tests for *H. pylori* infection, using the UBT as the gold standard, among symptomatic patients presenting with gastrointestinal complaints.

Study population and sampling

The study included all adult patients with symptoms consistent with dyspepsia. Participants were selected using a convenience sampling method based on their availability and willingness to participate. Prior to enrollment, all individuals were screened for recent proton pump inhibitor (PPI) and antibiotic use. Individuals who reported using PPIs or antibiotics within the two weeks preceding testing were not included in the study at all, due to the known suppressive effects of these medications on *H. pylori* detection.

A significant number of initially screened patients were excluded because of recent over-the-counter PPI use, which is highly prevalent in the local setting. This contributed to the final study sample size of 66 participants. Although a formal recruitment flow chart was not included, the exclusion criteria were applied uniformly to all screened patients to minimize diagnostic misclassification.

Eligibility Criteria

Inclusion criteria

a) Patients presenting to the family medicine OPD of Cure Hospital with clinical manifestations consistent with dyspepsia (epigastric pain, early satiety, postprandial fullness, bloating, and nausea) (21);

b) Adults who consented to undergo all three diagnostic tests, namely the UBT, stool antigen test, and serum antibody test; and patients who were able to provide a consent form.

Exclusion criteria

a) Patients with a history of receiving Proton Pump Inhibitors, including omeprazole, lansoprazole, or esomeprazole, in the last two weeks;
b) Patients who had taken oral or intravenous antibiotics effective against *H. pylori* infection, such as amoxicillin, clarithromycin, levofloxacin metronidazole, or tetracycline, within the last four weeks, as this could lead to false-negative results in the stool antigen test; and
c) Patients exhibiting alarm features indicative of potential gastrointestinal malignancy, specifically unintentional weight loss exceeding 3 kg within the last three months, dysphagia, odynophagia, hematemesis, or the presence of an abdominal mass.

Data collection

This study included 66 dyspeptic patients recruited over September to December 2023 at a tertiary hospital. Data collection was carried out by a team of trained medical doctors. Sociodemographic and clinical information, including medication history, was obtained through interviews with patients and their medical records.
Each participant provided blood, stool, and breath samples for diagnostic testing. The following procedures were employed for sample collection and analysis: Blood samples (4-5 ml) were collected in clot activator tubes, labeled, and checked for serum *H. pylori* antibody using a commercial ELISA kit. The ELISA method has demonstrated sensitivity of 75.75% and specificity of 45% in various studies (22), ensuring reliable assessment of *H. pylori* infection status.
Stool samples were collected in sterile containers and tested for *H. pylori* antigen using the *H. pylori* antigen detection kit. The test has shown sensitivity ranging from 85% to 95% and specificity between 90% to 98%, making it a reliable diagnostic tool (23).
For the UBT, patients fasted for at least six hours prior to the procedure. Breath samples for the level of CO_2 were collected before and 30

minutes after ingestion of a ^{13}C labeled urea-containing capsule. The increase in labeled carbon dioxide in the exhaled breath indicated a positive result, reflecting active *H. pylori* infection. The UBT is known for its high sensitivity of 92-100% and specificity of 87-100% validated by a systematic review (24).

The stool antigen test (Vaxpert, Inc., USA; 1HP-602) is a rapid immunochromatographic assay reported by the manufacturer to use monoclonal antibodies against *H. pylori* antigens. The serum antibody test (Healgen Scientific LLC; REF: RT21231) detects anti-*H. pylori* IgG antibodies and therefore reflects exposure rather than active infection. The urea breath test was the non-radioactive ^{13}C-UBT and explicitly stated its role as the reference standard for active *H. pylori* infection. Additional details of all assays are provided in the supplementary material (Table A).

The validity and reliability of these diagnostic tests have been established in various studies (25), supporting their use in assessing *H. pylori* infection among participants.

Blinding

All diagnostic tests (stool antigen test, serum antibody test, and urea breath test) were performed in the laboratory by trained laboratory personnel who were blinded to the patients' clinical information. Treating physicians were not aware of the laboratory test results at the time of patient assessment. This blinding minimized potential measurement and interpretation bias.

Study variables

H. pylori infection status, as determined by the UBT, was used as the gold standard reference for evaluating the accuracy of other tests.

Index tests consisted of serum antibody tests, stool antigen tests, and socio-demographic variables (age, sex, marital status, ethnicity, literacy level, and residence).

Statistical analysis

Data were entered into Microsoft Excel and analyzed using IBM SPSS version 21 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the characteristics of patients with gastrointestinal symptoms. Comparative analyses, including sensitivity, specificity, positive predictive value, and negative predictive value, were calculated to evaluate the diagnostic performance of stool antigen and serum antibody tests in comparison to the urea breath test. To evaluate the diagnostic accuracy of each test compared to UBT as the gold standard, Receiver Operating Curve (ROC) was used to estimate sensitivity and specificity of the tests as well as to calculate the Area Under the Curve (AUC) and overall diagnostic accuracy.

Ethical consideration

Prior to participant enrollment in the study, individuals were provided with detailed information regarding the study's objectives, methodologies, potential risks, benefits, and their right to withdraw at any time without facing any problems and repercussions. The administration of two other tests (UBT, Serum Ab) did not result in any additional financial burden for the patients, as all associated costs were covered by the research team.

To protect patient confidentiality, all individual patients' data was anonymized by removing identifying information during data collection and data analysis. Data were securely stored in a password-protected laptop. It should be noted that verbal consent was obtained from illiterate participants.

Results

A total of 66 participants were included in the study. The majority of participants were female (68.2%) and married (78.8%) (Table 1). Most of participants were aged between 18 and 30 years (39.4%), followed by those aged 31-45 years (36.4%). Regarding educational status, approximately half of the participants were literate and

half of them were categorized as illiterate. The majority of participants resided in urban areas (69.7%), and almost two-thirds of participants were unemployed. In terms of ethnicity, Pashtuns constituted one-third (33.3%) of participants.

The most common health complaint among participants was abdominal pain and discomfort, followed by early satiety, postprandial fullness, bloating, and nausea.

Table 1: Sociodemographic and clinical features of enrolled cases (N=66)

Variables	***Categories***	***N***	***%***
Mean Age ± SD	36.45 ± 13.23		
Sex	Male	21	31.8
	Female	45	68.2
Marital Status	Single	13	19.7
	Married	52	78.8
	Widowed, Divorced or others	1	1.5
Ethnicity	Pashtun	22	33.3
	Tajik	19	28.8
	Hazara	17	25.8
	Uzbek	2	3
	Others	6	9.1
Tobacco-related Habits	Smoking	3	4.5
	Snuff	4	6.1
	None	59	89.4
Education	Literate	35	51.5
	Illiterate	33	48.5
Employment	Employed	24	36.4
	Jobless	42	63.6
Residency	Urban	46	69.7
	Rural	20	30.3
Principle Complaints	Abdominal Pain and Discomfort	62	93
	Early Satiety	51	77.2
	Post Prandial Fullness	51	77.2
	Nausea	46	69.7
	Bloating	49	74.2
Diagnostic Tests Results	UBT		
	Positive	26	39
	Negative	40	61
	Stool Antigen Test		
	Positive	26	39
	Negative	40	61
	Serum Antibody Test		
	Positive	34	51
	Negative	32	49

The stool antigen test demonstrated a sensitivity of 96%, specificity of 97.5%, Positive Predictive Value (PPV) of 96%, and Negative Predictive Value (NPV) of 97.5% (Table 2). Diagnostic accuracy estimates were calculated using a total sample of 66 participants, comprising 26 UBT-positive and 40 UBT-negative cases. Detailed contingency data are presented in the supplementary material (Table B).

The sensitivity and specificity of serum antibody test were 73% and 67.5%, respectively. PPV of the test was 61% and NPV was 78% (Table 2). The serum antibody analysis was conducted on a total sample of 63 participants, including 26 UBT-positive and 37 UBT-negative cases. Three UBT-negative participants were excluded due to missing serum antibody results. Detailed information is provided in the supplementary material (Table C).

Table 2: Comparison of Stool Antigen and Serum Antibody Test Accuracy Against Urea Breath Test in Detecting *H. Pylori*

Variable	**Urea Breath Test (UBT)**			
Stool antigen test	**Positive**	**Negative**	**Row Total**	**Predictive Values**
Positive	25	1	26	PPV***=25/26 (96%)
Negative	1	39	40	NPV****=39/40 (97.5%)
Column Total	26	40		
	SN*=25/26 (96%)	SP**=39/40 (97.5%)		
	Urea Breath Test (UBT)			
Serum Antibody Test	**Positive**	**Negative**	**Row Total**	**Predictive Values**
Positive	19	12	31	PPV=19/31 (61%)
Negative	7	25	32	NPV=25/32 (78%)
Column Total	26	37		
	SN=19/26 (73%)	SP= 25/37(67.5%)		

*SN (Sensitivity)= Number of correctly diagnosed positive/Number of total tested positive (including false negative)
**SP (Specificity)=Number of correctly diagnosed negative/Number of total tested negative (including false positive)
***PPV (Positive Predictive Value) = Number of true positive tests/Total number of positive tests (including false positive)
****NPV (Negative Predictive Value) = Number of true negative tests/Total number of negative tests (including false negatives)

Considering sex-based diagnostic performance, the stool antigen test among male participants (n = 21) had perfect performance, with 100% sensitivity and specificity (7 true positives and 14 true negatives). Also, the stool antigen test among female participants (n = 45) performed very well, with a sensitivity of 94.7% and specificity of 96.2%. No statistically significant difference was observed between males and females for stool antigen test positivity (P = 0.88). Similarly, differences in serum antibody test performance by sex were not statistically significant (P = 0.52).

The results of the serum antibody test showed lower accuracy, with a sensitivity of 57.1% and specificity of 64.3% among males, and the sensitivity and specificity of antibody test among females were 78.9% and 61.5%, respectively. Detailed sex-specific diagnostic performance data are provided in the supplementary material (Table D).

The ROC curve for the stool antigen test (Figure 1) indicated near-perfect diagnostic performance, with the curve approaching the upper left corner, consistent with high sensitivity and specificity values. The Area Under the Curve (AUC) for Stool Antigen test against UBT was found to be 0.968 (95% CI: 0.917–1) with P <0.001, illustrating statistical significance. The overall accuracy of this test was 96.9%.

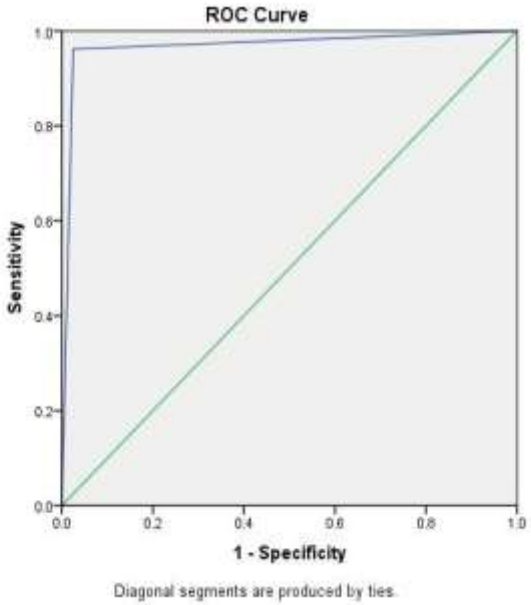

Figure 1: ROC curve of stool antigen test for detection of *H. pylori* infection compared to Urea Breath Test

Conversely, the ROC curve for the serum antibody test (Figure 2) showed lower diagnostic accuracy of 69.8%. with an AUC of 0.678 (95% CI: 0.545-0.811) and a *P* -value of 0.015, indicating statistical significance but inferior discriminatory ability compared to the stool antigen test.

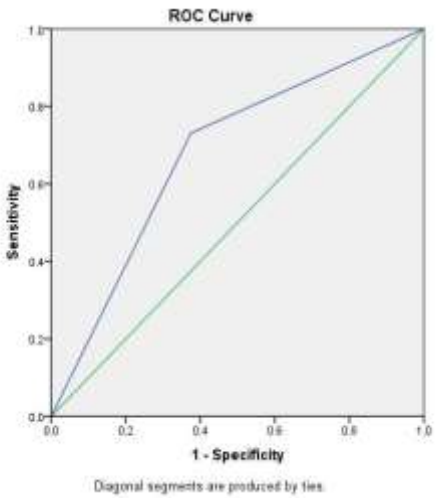

Figure 2: ROC curve of serum antibody test for detection of H. Pylori infection compared to urea breath test

Discussion

This study compared the diagnostic performance of the stool antigen test and the serum antibody test against the UBT, which serves as the reference standard for detecting *H. pylori* infection among symptomatic patients in Kabul, Afghanistan. The stool antigen test showed diagnostic

accuracy, with high sensitivity, specificity, and overall accuracy was near to UBT.

One of the key advantages of the stool antigen test is its ability to detect active infection rather than past exposure, which is particularly beneficial in settings where IgG antibodies may remain elevated long after infection has cleared. This characteristic makes the stool antigen test a practical and reliable alternative to UBT, especially in areas where UBT is inaccessible or unaffordable.

Our results are very similar to those of Gisbert et al.'s large meta-analysis, which found that monoclonal stool antigen tests had a pooled sensitivity of 94% (95% CI 93-95%) across multiple studies (26). Similarly, in a large-scale meta-analysis focusing on pediatric populations, Zhou et al. reported pooled sensitivity of 92.1% and specificity of 94.1% for stool antigen tests, thereby confirming the efficacy of this method across various age groups (27). This emphasizes the effectiveness of stool antigen test for detecting *H. pylori* infection.

Regarding serology, our study's serum antibody test had results (sensitivity 73%, specificity 67.5%, AUC 0.678) similar to those from a pediatric meta-analysis by Yu et al., which reported that the pooled sensitivity for various IgG ELISA tests was 79.2% (28) and the specificity was 89% (29). These values are lower than serum antibody values, which is a well-known limitation of serum antibody tests. This is especially true in settings with high prevalence and chronic exposure, where serology can overestimate active infection (30).

Our findings also revealed gender-based differences in the diagnostic performance of both tests. For the antibody test, females had higher sensitivity (78.9%) compared to males (57.1%), suggesting better ability to detect infection among women. Conversely, specificity was slightly higher in males (64.3%) than females (61.5%), indicating that a negative antibody result might be more reliable in ruling out infection among male patients. Obaidd et al. also demonstrated notable differences in the sensitivity and specificity of antibody tests based on gender, revealing superior specificity in males and enhanced sensitivity in females (31).

These differences may be influenced by several factors, including biological variation in immune response, healthcare-seeking behavior, or environmental exposure patterns (32), particularly relevant in Afghanistan. However, due to the limited sample size (particularly among males) these differences should be interpreted with caution.

The ROC curve for the stool antigen test neared the upper-left corner, indicating near-perfect discrimination, while the serum antibody curve was significantly lower, with an AUC of 0.678. These findings are alongside with a meta-analysis of diagnostic accuracy test and a cross sectional study from Mardan, Pakistan (33,34) in clinical settings, highlighting reliance on inappropriate treatment decisions, which resonates with concerns identified in other studies.

In Afghanistan, where access to UBT and endoscopic diagnostics is limited-particularly in rural areas-the stool antigen test provides a practical, accurate, and non-invasive alternative.

The UBT, while accurate, requires specialized equipment and trained personnel and is not widely available outside major urban centers. Beside these challenges the average cost of UBT is about $17.50 USD, in comparison with stool antigen at $4.35 USD and antibody test at $2.17 USD, so it shows the affordability of the UBT test is difficult. In contrast, the stool antigen test is cost-effective, easy to administer, and does not require advanced laboratory infrastructure, making it a practical choice for both urban and rural health facilities. Given the high prevalence of *H. pylori* infection reported in Afghanistan-up to 75% in some regions-wider adoption of stool antigen testing could improve diagnostic accuracy, support targeted therapy, and reduce the long-

term burden of *H. pylori*-associated complications such as peptic ulcer disease and gastric cancer.

Limitations

The current study was subject to several limitations. Firstly, the number of participants in this study was small, which limits the generalizability of our findings. However, the widespread Over-the-Counter (OTC) use of PPIs among the study population played a key role in this small number of participants, since the majority of the referred patients used PPIs in the last two weeks prior to the study, thus rolling them out of the study. Secondly, the study setting was limited to one health facility, which also reduces the generalizability of the study findings. Additionally, only a single commercial kit for both the stool antigen and serum antibody tests, without comparison to alternative brands or platforms, the generalizability of our diagnostic accuracy estimates may be limited, as kit-to-kit performance, variability is well documented.
Finally, the study relied on patient self-report to determine PPI use and antibiotics use eligibility for inclusion, which introduces the potential for recall bias.

Conclusion

The stool antigen test demonstrated high diagnostic accuracy compared with the urea breath test, indicating that it is a reliable and accessible tool for identifying *H. pylori* infection in our setting. In contrast, the serum antibody test showed lower performance, limiting its usefulness for routine diagnosis.
These findings highlight the value of stool antigen testing as a feasible option for frontline clinical practice. We recommend that the stool antigen test be considered for integration into national diagnostic protocols and clinical guidelines, particularly in resource-limited environments where access to UBT is constrained. Future studies comparing multiple kits would further strengthen the evidence for national adoption.

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Conflict of interests

The authors declare they have no conflict of interests.

Availability of data and materials

Supplementary data are available upon request from the corresponding author based on reasonable request.

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