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Original Research Article

Is rapid urease test as reliable as histopathological evaluation for *Helicobacter pylori* Infection- A prospective study?

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ABSTRACT

Background: *Helicobacter pylori* (*H. pylori*) is a widespread pathogen causing gastroduodenal damage. The Rapid Urease Test (RUT), a quick, affordable, and simple diagnostic tool, is commonly used to detect urease presence in the gastric mucosa. Given *H. pylori*'s global prevalence, accurate diagnosis is crucial. Our study aims to compare the accuracy of RUT with histopathology, and the effectiveness of Hematoxylin & Eosin (H&E) versus Modified Giemsa (MG) staining in detecting *H. pylori*.

Materials and Methods: In this study conducted from October 2022 to January 2024, the Rapid Urease Test (RUT) results were juxtaposed with the histopathological findings of gastric biopsies. These biopsies were undertaken in the Department of Pathology at MMIMSR. The biopsy samples underwent routine tissue processing and were subsequently stained with H&E and MG. The histological analysis was carried out following the guidelines of the modified Sydney system.

Results: Gastric biopsies from 105 patients were analyzed using RUT, Hematoxylin & Eosin (H&E), and MG staining. The results showed that 59.05% of patients tested positive for *H. pylori* using RUT, and 60% tested positive on histopathology. The sensitivity and specificity of RUT were 82.54% and 76.19%, respectively, with a significant association between RUT and Histology (p-value <0.0001, Chi-square test =35.95). The sensitivity and specificity of H&E staining were 79.37% and 100.00%, respectively.

Conclusion: The study concluded that RUT is a reliable initial detection method for *H. pylori*, but negative results should be confirmed with histological evaluation using H&E and MG stain.

1. Introduction

Helicobacter pylori (*H. pylori*) is a significant global health concern, causing severe illnesses like peptic ulcer disease and stomach cancer. *H. pylori* continues to be widespread, particularly in areas with poor resources, potentially infecting nearly half of the global population.[1]

In 1893, Italian researcher Giulio Bizzozero described a spiral bacterium, spirilla, found in dog's stomachs. In 1899, Walery Jaworski noted bacteria in the human stomach, hypothesizing their link to stomach ulcers and gastric cancer. The significance of these bacteria, later identified as *H. pylori*, was recognized in the late 1970s when J.R. Warren observed their presence in inflamed gastric mucosa[2] following which in 1983 Warren and Marshall successfully cultured this spiral organism and received a Nobel prize in 2005.

H. pylori can persist in the stomach's acidic environment due to its ability to form a protective biofilm; this results in prolonged colonization, inflammation, and tissue damage.[3] Although *H. pylori* infection frequently leads to chronic gastritis, in some individuals it can result in peptic ulcer disease, non-cardiac gastric cancer, and MALT (mucosa-associated lymphoid tissue) lymphoma.

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To diagnose *H. pylori* infection, multiple tests are used. Invasive methods include the Rapid Urease Test (RUT) and histology, performed during upper gastrointestinal endoscopy. Non-invasive tests include the Urea Breath Test (UBT) and Stool Antigen Tests (SATs), which previously used an enzyme immunoassay.

2. Materials and Methods

105 patients with Upper Gastrointestinal (UGI) symptoms underwent endoscopy at MMIMSR in this prospective study. At least, two gastric antral mucosal biopsies were taken from each patient and subjected to the Rapid Urease Test (RUT). The study included dyspepsia patients and patients with other Upper Gastrointestinal (UGI) symptoms not on proton pump inhibitors or antibiotics. Patients with a history of confirmed biopsies, symptomatic cholelithiasis, disturbed gastric physiology, pregnancy, breastfeeding, unwillingness for endoscopy/biopsy, non-compliance, or age less than 18 years were excluded from this study. The results of RUT were compared with histopathological findings from October 2022 to January 2024. Biopsy samples were tested immediately with a rapid urease kit, fixed in formalin overnight, and then processed and stained with H&E and MG for detailed histopathological analysis. The results of RUT were observed for at least an hour.

3. Results

Gastric biopsies were collected from 105 patients presenting with complaints of dyspepsia and other upper GI symptoms consecutively from October 2022 to January 2024 to measure the efficacy of the Rapid urease test using histopathology as a gold standard method. Out of these cases, 61(58.10%) were and 44(41.90%) were females, with male to female ratio of 1.4:1. Their ages ranged from 18 years to 77 years with a mean age of 42.01±14.76 with the youngest and oldest patient being a female.

Among the various endoscopic findings of these patients, 87(82.8%) had antral hyperemia and among them, 44(50.57%) were associated with *H. pylori* as given in Table 1. The rest of the findings were pangastritis, gastropathy, polyp, gastric mucosal ulcers, gastric erosion, and esophagitis with their percentage association with *H. pylori*.

Among various other symptoms, dyspepsia 86(81.90%) was the major upper GI symptom and remaining were Loss of Weight and Loss of Appetite 3(2.86%), Melena 6(5.71%), Gastric Reflux 2(1.90%), Pain Abdomen 5(4.76%), Hematemesis 1(0.95%), Dysphagia 1(0.95%), Chronic Diarrhea 1(0.95%) with Dyspepsia 1(0.95%).

Among the total number of cases, 52 were true positive, 32 were true negative, 10 were false positive and 10 were false negative (Table 2).

Table 1: Correlation of upper GI endoscopic findings with *H. pylori*

Endoscopy results		Patients (N, %)	*H Pylori* Detected (N, %)
Antral hyperemia		87(82.8%)	44(50.57)
Pangastritis		9(8.5%)	7(77.78)
Gastropathy		8(7.6%)	6(75)
Polyp		1(0.9%)	0(0)
Gastric mucosal ulcer		5(4.7%)	3(60)
Gastric Erosions		2(1.9%)	1(50)
Esophagitis	LA Grade A	4(3.8%)	1(25)
	LA Grade B	4(3.8%)	2(50)

Table 2: Association of Rapid urease test with the histopathological detection of *H. pylori*

	Histopathological Detection of *H. pylori*		
Rapid Urease test status	**Detected (N, %)**	**Not detected (N, %)**	**Total**
Positive	52 (49.52%)	10 (9.52%)	62 (59.05%)
Negative	11 (10.47%)	32 (30.48%)	43 (40.95%)
Total	63 (60%)	42 (40%)	105(100%)

The sensitivity and specificity of the rapid urease test are 82.54% and 76.19% the Positive predictive value = 83.87%, the Negative predictive value = 74.42% and the predictive validity of the rapid urease test is 80.00%.

Table 3 indicates that a higher density of *H. pylori* is linked to more severe gastric mucosal inflammation, with the majority of moderate and severe cases showing high bacterial density.

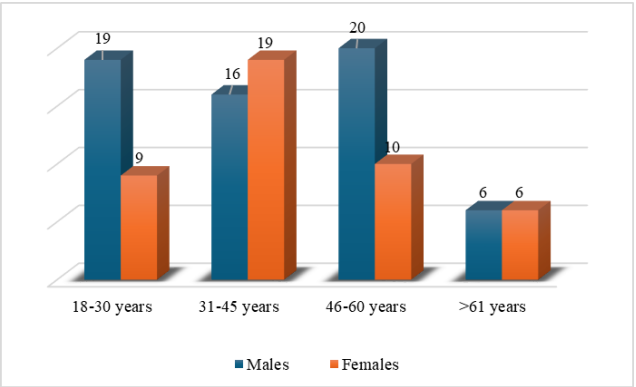

Figure 1: Represents age and gender distribution

Table 4 displays data about the severity of inflammation with the presence of *H. pylori*. There was a total of 105 instances, with 58.10% being male and 41.90% being female. The H&E staining method revealed that 47.62% of the samples tested positive for *H. pylori*, but the MG staining method showed a positivity of 60% for *H. pylori*.

Table 3: Correlation between the Density of *H. pylori* and Severity of gastric mucosal inflammation

Density of *H. pylori*	Severity of Inflammation			Total
	Mild	Moderate	Severe	
0	31 (65.96%)	12 (22.22%)	0 (0%)	43 (40.95%)
1	11 (23.40%)	19 (35.19%)	0 (0%)	30 (28.57%)
2	05 (10.64%)	23 (42.59%)	3 (75%)	31 (29.52%)
3	0 (0%)	0 (0%)	1 (25%)	1 (0.95%)
Total	47 (100%)	54 (100%)	4 (100%)	105 (100%)

Table 4: Distribution of the 105 patients according to the intensity of inflammation of gastric mucosa, and H. pylori positive and negative staining results by MG and H&E methods

Intensity of Inflammation	N, %	*H. pylori* Status			
		H&E		MG	
		HP Positive	HP Negative	HP Positive	HP Negative
Mild	46 (43.81%)	7 (15.22%)	39 (84.78%)	16 (34.78%)	30 (65.22%)
Moderate	55 (52.38%)	39 (70.91%)	16 (29.09%)	43 (78.18%)	12 (21.82%)
Severe	04 (3.81%)	04 (100%)	00 (0%)	04 (100%)	00 (0%)
Total	105 (100%)	50 (47.62%)	55 (52.38%)	63 (60%)	42 (40%)

Table 5 shows that out of 105 cases, 60% were positive for *H. pylori* with Modified Giemsa stain, while only 40% were positive with H&E stain. Half of the cases were positive with both stains, and a small percentage were positive with H&E but negative with MG.

Statistical analysis of the agreement between H&E and MG stain for the histopathological detection of *H. pylori* (chi-square = 63.64), P< 0.001. The sensitivity and specificity of the H&E staining are 79.37% and 100.00%, the Positive predictive value = 100.00%, the Negative predictive value = 76.36%, and the predictive validity of the rapid urease test is 87.62%.

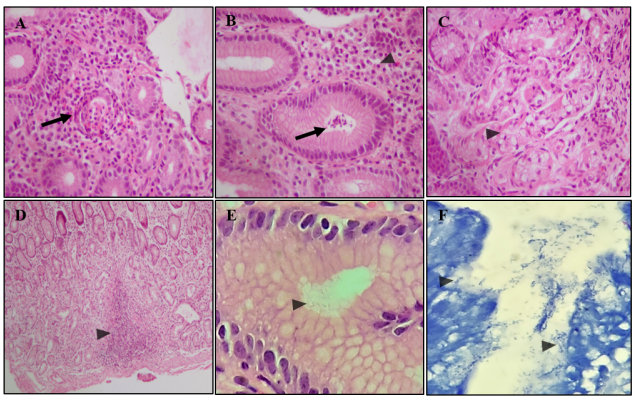

Figure 2: Photomicrographs (H&E, 400x) (**A & B**): depict focal activity (arrow) and moderate mononuclear infiltrates (arrow head) within the Antral mucosae; **C):** (H&E, 400x) shows intestinal metaplasia with goblet cells (arrow head). **D)**: Shows chronic antral gastritis with mild glandular atrophy and lymphoid follicle; **E and F):** (H&E, 100x and MG, 1000x), show *H. pylori* bacilli (arrow head) clinging to the gastric epithelium

4. Discussion

To accurately diagnose *H. pylori* infection, we need tests with over 90% specificity and sensitivity. The gold standard is histopathological analysis of gastric biopsies. A cost-effective alternative is the RUT.[4] This test uses the bacteria's ability to produce urease, which breaks down urea into ammonia and carbon dioxide, causing a color change in a pH indicator solution. Both rapid urease testing, and histopathology are used to diagnose *H. pylori* infection, depending on the patient's condition, available resources, and the specific clinical context. Importantly, these tests can complement each other in clinical practice.

The youngest patient in our study was 18 years old, and the oldest patient was 77 years old with a mean age of 42.01±14.76 years. The mean age group from our study was similar to the studies by Losurdo et al.,[5] Yakoob et al.[6] and Choi et al.[7]

58.1% male and 41.9% female patients with a ratio of 1.4:1 was part of this study. This data corresponded to studies by Yakoob et al.,[6] Buharideen et al.,[8] Dechant et al.,[9] and Archimandritis et al.

From the results, 59.05% were positive for rapid urease test and 60% positive on histopathology. This was in concordance with the studies done by Chi-Chen Fan et al.[10], Archimandritis et al.,[11] Yakoob et al.[6] and Karki et al.[12] with a slightly higher positivity rate in our study(59.05%). The study by Athavale et al[13] showed a higher positivity rate of RUT (84%) which was slightly higher than the positivity rate of histopathology (83%).

Higher sensitivity (82.5%) of the rapid urease test was observed in our study which was also observed in studies done by Chi-Chen Fan et al.,[10] Karki et al.,[12] and Athavale et al.[13] While, studies done by Archimandritis et al.[11] and Yakoob et al.[6] show much lower sensitivity (64% and 71.9%

Table 5: Association between H&E stain and MG stain for histopathological detection of *H. pylori*

	***H Pylori* Detected on MG Stain**		
***H Pylori* Detected on H&E**	**Detected (N, %)**	**Not detected (N, %)**	**Total (N, %)**
Detected (N, %)	50 (47.62%)	0 (0%)	50 (47.62%)
Not detected (N, %)	13 (12.38%)	42 (40%)	55 (52.38%)
Total	63 (60%)	42 (40%)	105(100%)

respectively). A large gap was noted in our study in terms of specificity, as it was concordant with Athavale et al[13] (74.4%) and Yakoob et al.[6] (80%) but was much higher in the study by Chi-Chen Fan et al.[10] (100%), Archimandritis et al.[11] (93%) and Karki et al.[12] (94.4%).

The accuracy of the Rapid Urease Test (RUT) for *H. pylori* depends on bacterial concentration, biopsy location, and patient factors like recent proton pump inhibitor use. Optimal sampling includes antrum and corpus regions, avoiding metaplastic areas to reduce false negative results.[6,14]

False positives in RUT for *H. pylori* are rare, mainly due to other urease-producing bacteria, especially in low stomach acid environments.[15,16]

All of the received gastric biopsies were processed and stained with H&E stain and were reported to be chronic gastritis as per the updated Sydney System.[17,18] All the biopsies showed chronic inflammation in the mucosa. Mononuclear infiltrates were graded into absent, mild, moderate, and severe which were 0%, 43.8%, 52.3%, and 3.8%, respectively.

In our study, 8.5% of cases showed chronic inflammation with activity, with *H. pylori* detected in 88.8% of these cases. This is similar to the findings of Hassan et al.[19] and Sharma et al.[20] All of the cases of mucosal atrophy (1.9%) in our study were positive for *H. pylori* which was much lower in comparison with studies of Hassan et al,[19] Sharma el al[20] and S. Boldt et al.[21]

In the study, the higher density of *H. pylori* is associated with grade 2 and 3 chronic inflammatory infiltrates which is in concordance with studies done by Peng et al.,[22] Basir et al.[23] and Serhat Sayin.[24] Additionally, our work demonstrates a clear correlation between the intensity of *H. pylori* infection and the extent of chronic gastritis. Therefore, individuals with mild colonization had a significantly lower likelihood of developing severe chronic gastritis compared to those with severe colonization, where the possibility was approximately 25%. However, this relationship was not seen in a study undertaken by Park et al.[25] in Korea. This disparity could be attributed to genetic variances, eating preferences, and environmental factors in the study populations.

Another aim of our study was to correlate H&E and MG stain on histopathology for the detection of *H. pylori*. In our study, the detection rate of *H. pylori* with H&E was much lower (47.6%) as compared to the MG stain (60%). Our study was supported by the similar positivity rates in studies by S. Boldt et al.,[21] Priyadarshini et al.,[26] García-Carmona et al,[27] and Nadeem et al.[28]

The sensitivity, specificity, PPV, and NPV of H&E stain in our study are 79.3%, 100%, 83.8%, and 74.4% respectively. Our study was in concordance with Nadeem et al.,[28] García-Carmona et al[27] and S. Boldt et al.[21] in terms of sensitivity and specificity. A significant association was observed between the H&E stain and MG stain with a p-value of 0.001, a Chi-square ($\chi 2$) value equal to 63.6%, and a predictive validity of 87.62%.

Apart from the conventional H&E stain, several sets of stains have been suggested, such as Giemsa, PAS-AB, Warthin-Starry, and IHC stains.[29] Nevertheless, several authors recommend auxiliary testing for instances where there is a strong suspicion of *H. pylori* infection that cannot be seen visually with H&E staining, such as cases with active gastritis or germinal center formation.[30]

Giemsa is the most widely used special stain and the accepted gold standard for the histopathological detection of *H. pylori* because it is inexpensive, readily available, and simple to perform. The only downside is the absence of contrast between the bacilli and the surrounding tissue.[31]

While some writers suggest the regular utilization of immunohistochemistry (IHC) stains to detect *H. pylori*,[32] but, because their application requires a significant amount of time and money most authors suggest their usage only when necessary.[29]

5. Conclusion

Histopathologic testing and the Rapid Urease Test (RUT) are both reliable for diagnosing *H. pylori* in dyspeptic patients. RUT is cost-effective and easy to perform, but it should be used as a preliminary test. False negatives are more common than false positives, so a negative RUT result doesn't necessarily rule out *H. pylori*, especially in clinically suspected cases. If RUT results are negative, consider histopathological evaluation using H&E and MG stain for *H. pylori* detection.

6. Source of Funding

None.

7. Conflicts of Interest

The authors declare no conflicts of interest.

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