

Reliability of RUT done on endoscopy guided gastric biopsy in detecting Helicobacter Pylori

Wasif Mohammad Ali¹, Khadeeja Tanveer², Asiya Zehra¹, Parkar Zainuddin Liyakat², Nishat Afroz³

Abstract

Introduction: This study was done to identify the reliability of RUT in detecting H.Pylori in patients of gastritis by performing Histopathological examination. Endoscopic biopsy samples were taken from gastritis lesions present in the stomach.

Methods: This was a prospective observational study done at Jawaharlal Nehru Medical College (JNMC), Aligarh Muslim University (AMU), Aligarh between May 2021 and May 2022 with a sample size of 230 patients. The endoscopists performed RUT on biopsy samples taken from gastritis lesions and sent another biopsy sample from the same patient for histopathological examination. On the basis of histopathological report, results were compared and analyzed.

Results: In our study the specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of RUT were 60.81%, 67.31% ,78.36%, 46.88% , and 65.22%, respectively.

Conclusion: Our study suggests that RUT can reliably identify Helicobacter pylori infection in gastric mucosa.

Keywords: Endoscopy; Helicobacter Pylori; Rapid Urease Test.

Author affiliations:

¹Department of Surgery, Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh, Uttar Pradesh, India 202002.

²Department of Surgery, F.H. Medical College and Hospital, Agra, Uttar Pradesh, India 283204.

³Department of Pathology, Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh, Uttar Pradesh, India 202002.

Correspondence:

Dr. Khadeeja Tanveer,
Department of Surgery, F.H. Medical College and Hospital, Agra, Uttar Pradesh, India 283204.

Email: khadeejatanveer2k13@gmail.com

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Introduction

Helicobacter pylori is a gram-negative helical bacteria that dwells in the antral mucosa, specifically at the interface between surface stomach epithelial cells and the mucous gel layer above them.¹ *H. pylori* infection can cause a variety of symptoms, from asymptomatic gastritis to gastrointestinal cancer. Antral-based gastritis affects around 95% of infected persons and puts them at risk for duodenal ulcers; therefore duodenal ulcers are also very common. Corpus-predominant gastritis is more common and puts people at risk for stomach ulcers. More than 90% of duodenal ulcer and about 75% of all peptic ulcer patients have *H. pylori* infection. The chance of ulcer recurrence is greatly reduced when the bacterium is eradicated.² With *H. pylori* infection, the lifetime risk of peptic ulcer disease is 20%, and the risk of gastric cancer is 1–2%.³ Infection with *Helicobacter pylori* can range from 14 percent in developed countries to 92 percent in developing countries.^{4,5}

H. pylori is urease, catalase and oxidase positive. The urease activity is striking, and the amounts produced have allowed accurate diagnosis in patients by direct detection of the enzyme in gastric biopsy specimens and by breath tests using carbon isotopes labeled with urea. Many roles have been proposed for urease enzymes. It is known to be important for colonization and survival of the bacterium in the gastric environment.⁶ The hydrolysis of urea to ammonia by urease could have a buffering effect, protecting the bacterium from acidity.⁷ In vitro studies have shown that *Helicobacter pylori* cannot survive in acidic condition without the presence of urea, and urea inhibits its growth in alkaline conditions.⁸⁻¹⁰ Urease also has been proposed as an important virulence factor. The urease activity of *H. pylori*, once it enters the human stomach, neutralizes the acidic state that existed at the start of infection. *H. pylori* then travels toward host gastric epithelial cells with the help of flagella, where precise interactions between bacterial adhesins and host cell receptors result in effective colonization and permanent infection. *H. pylori* has developed an acid acclimation system that regulates urease activity to promote periplasmic pH adjustment in the severe acidic environment of the stomach. *H. pylori* requires intra bacterial urease activity for acid resistance, and this activity is controlled by the proton-gated urea channel UreI, which allows urea entrance only under acidic conditions to avoid deadly alkalization during times of relative neutrality. Phagocytosis is a part of the innate immune system that helps to eliminate invading microorganisms. Urease regulates the pH of phagosomes and the development of megasomes, making it crucial for *H. pylori* survival in macrophages. Only endoscopy with numerous biopsy specimens collected from one or more regions of the stomach, including the antrum, body, and transition zone (cardia and body), can reliably diagnose *H. pylori* and show evidence of infection effects.¹¹ The resulting sample can be used to test urease activity, histology, and/or PCR. Here in this study we are more interested in RUT as it is low priced, easy to use, and rapid time to diagnosis. RUT is very helpful and valuable to the patients as it gives

a positive result for *H. pylori* infection before the patient leaves the endoscopic suite.

Histopathological Examination of Biopsy Tissue

With specificity and sensitivity rates surpassing 90%, Hematoxylin and Eosin, modified Giemsa, and Warthin-Starry stains can detect *H. pylori* in the stomach mucosa.¹² Due to sampling mistake or patchwork distribution of the lesion, false negative results can occur.¹³ Gastric antral biopsies were obtained in a study, and several stains such as Hematoxylin and Eosin, Giemsa, Brown Brunn, Warthin Starry, and Brown Hopps were used. The experts concluded that Wright Giemsa was the easiest to conduct and that technique should be used routinely for *H. pylori* detection in antral biopsy.¹⁴

Methods

This study was conducted at Jawaharlal Nehru Medical College (JNMC), Aligarh Muslim University (AMU), Aligarh between May 2021 and May 2022 with a sample size of 230 patients.

In our institute all patients undergoing endoscopy due to gastritis have to go through RUT and Histopathological examination. The patients were asked to report in the endoscopy suite nil per oral for six hours and accompanied by some responsible attendant. Informed and written consent was obtained and the patient was taken up for upper GI endoscopy. Biopsy was sent to pathology department in sterile Normal Saline, samples were taken from suspicious areas as erosion, ulceration, hyperemia etc. On the other hand RUT test was done using RUT DRY Test kit manufactured and marked by GASTRO CURE SYSTEMS VADODARA, INDIA. Color change from yellow to pink denoted a positive urease test (RUT positive).

Statistical analysis

The presentation of the Categorical variables was done in the form of number and percentage. The association of the variables which were qualitative in nature were analyzed using Chi-Square test. Sensitivity, specificity, positive predictive value and negative predictive value was assessed of NBI grading and RUT for predicting *H. pylori* positive and severe grade at each cut off point. The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 21.0. For statistical significance, p value of less than 0.05 was considered statistically significant.

Inclusion Criteria:

All patients >14 years of age having symptoms of gastritis or gastritis diagnosed on Upper GI Endoscopy at JNMC, A.M.U. Aligarh.

Exclusion Criteria:

Patients previously diagnosed as a case of Helicobacter Pylori gastritis and received the anti H. Pylori treatment.

Results

The study was conducted in J.N Medical College, AMU, Aligarh from May 2021 to May 2022. 230 patients of age >14 years having gastritis were included in the study. Intra Procedure monitoring was done. Endoscopic Findings noted and biopsy taken from gastritis lesions, RUT performed results noted and histopathological status of *H. Pylori* were noted.

Table 1. Distribution of RUT of study subjects.

RUT	Frequency	Percentage
Negative	74	32.17%
Positive	156	67.83%
Total	230	100.00%

In majority [156(67.83%)] of patients, RUT was positive. RUT was negative in only 74 out of 230 patients (32.17%) (Table 1).

Table 2. Distribution of H pylori status on histopathology of study subjects.

H pylori status on histopathology	Frequency	Percentage
H. pylori -	96	41.74%
H. pylori +	134	58.26%
Total	230	100.00%

In majority (134(58.26%)) of patients, *H. pylori* status on histopathology was positive. *H. pylori* status on histopathology was negative in only 96 out of 230 patients (41.74%) (Table 2).

Table 3. Association of H. pylori status on histopathology with RUT.

H. pylori status on histopathology	RUT Negative (n=74)	RUT Positive (n=156)	Total	P value
H. pylori -	45 (60.81%)	51 (32.69%)	96 (41.74%)	<0.0001†
H. pylori +	29 (39.19%)	105 (67.31%)	134 (58.26%)	
Total	74 (100%)	156 (100%)	230 (100%)	

† Chi square test

Proportion of patients with *H. pylori* status on histopathology:- negative was significantly higher in negative RUT as compared to positive RUT. (*H. pylori* - =60.81% vs 32.69% respectively). Proportion of patients with *H. pylori* status on histopathology:- positive was significantly lower in negative RUT as compared to positive RUT. (*H. pylori* + =39.19% vs 67.31% respectively). (p value <0.0001) (Table 3).

Among the patients who had *H. pylori* positive status, 67.31% of patients had positive RUT. If RUT was positive, then there was 78.36% probability of *H. pylori* positive status and if RUT was negative, then 46.88% chances of *H.*

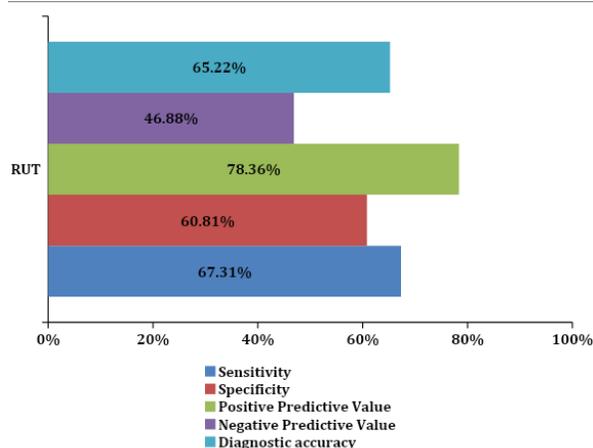


Figure 1. Sensitivity, specificity, positive predictive value and negative predictive value of RUT for predicting *H. pylori* positive status on histopathology.

pylori negative status. Among patients who had negative *H. pylori* status, 60.81% of patients had negative RUT (Figure 1).

Discussion

RUT is an indirect test for the presence of *H. pylori* based on the presence of urease in or on the stomach mucosa. It has a distinct advantage over serology in that it only detects current infection. This test is based on *H. pylori* producing urease, which catalyzes a chemical reaction in which urea is digested to produce CO₂ and ammonium ions. If the organism is present, material acquired by endoscopic biopsy changes color from yellow to red when placed in an appropriate medium.¹⁶ When the pH rises, phenol red, a color indicator that shifts from yellow to pink or red, is used in the test. This is visible to the naked eye. McNulty and Wise first reported this test in 1985, and numerous commercial preparations such as CLO, OMERT,¹⁷ and Christensen's medium are available, with results ranging from a few minutes to 24 hours. Commercially available RUT kits suggest that the decision be made (positive vs. negative) within 24 hours. Most will turn positive within 120 to 180 minutes but it is best to hold those that appear negative for 24 hours.^{18,19} After 24 hours the test may turn positive from the presence of non-*H. pylori* urease containing organisms.²⁰ Positive results after 24 hours are most often false positive and should not be used for treatment decisions. It has been postulated that blood leads to decrease sensitivity of RUT possibly related to the presence of albumin,²¹ *H. pylori* killing factors in human plasma,²² or blood in gastric lumen,^{23,24} however, other studies report that blood does not influence the test.^{25,26} False negative tests are also common after partial gastrectomy probably because of reduced bacterial load often related on the presence of bile.^{27,28}

In our study the specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), and

diagnostic accuracy of RUT were 60.81%, 67.31%, 78.36%, 46.88%, and 65.22%, respectively. The study done by Roy et al²⁹ where the specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of RUT were 97.22%, 94.04%, 98.75%, 87.5%, and 95%, respectively. In a study done by Kazemi et al³⁰ the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy 93%, 75%, 95%, 94%, 86% respectively. In a Study done by Karki et al³¹ the sensitivity, specificity, PPV, NPV, accuracy of RUT of H. Pylori were 84.1%, 94.4%, 94.9%, 82.9%, 88.8%.

Conclusion

In conclusion, we consider RUTs to be cheap and fast alternative to histology in patients with endoscopic signs of gastritis. Histological evaluation although gold standard is expensive, time consuming and may be unnecessary in some cases.

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