

Combined Rapid Urease Test and Histology For The Diagnosis of *Helicobacter Pylori* Infection



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ABSTRACT

Significance: Accurate detection of *Helicobacter pylori* (HP) is essential for the diagnosis of HP infection. The use of antibiotics and proton pump inhibitors (PPI) may give a false-negative rapid urease test (RUT) result. It is, therefore, suggested that histologic examination be done in combination with RUT. We aimed to determine the sensitivity and specificity of RUT compared with histology and assess the detection rate of combined RUT and histology for HP infection.

Methodology: Retrospective data collection was performed on 192 patients who were tested for both RUT and histology at the time of esophagogastroduodenoscopy (EGD) from 2017 to 2018. At least two gastric biopsies, one from the corpus, one from the antrum, were taken for both RUT and histology. EGD was performed by a single gastroenterologist. A single pathologist was responsible for interpreting the histology with hematoxylin and eosin (H&E) and Giemsa stain. The gold standard test for the diagnosis of HP infection was histology. Demographic profile, RUT and histology results were reviewed. Tests for diagnostic accuracy were computed using SPSSv23.

Results: One hundred ninety two (192) patients were tested for RUT and histology. Fifty two (27.1%) were males and 140 (72.9%) were females, with a mean age of 54 ± 17 years. Epigastric pain was

the most common indication for EGD seen in 42.7% of patients. Twenty four (12.5%) patients tested positive for HP infection by histology. Among these, 16 (8.3%) tested positive for both RUT and histology (true-positive), while 8 (4.2%) tested negative for RUT but had positive histology (false-negative). Six out of 8 (75%) patients with false negative results had PPI use. The sensitivity and specificity of RUT for the diagnosis of HP infection were 66.7 and 98.2%, respectively. While the positive and negative likelihood ratio were 37.3 and 0.34, respectively, with a diagnostic odds ratio of 110.

Conclusion: The sensitivity and specificity of RUT for the diagnosis of HP infection were 66.7 and 98.2%, respectively. The addition of histologic examination to RUT increased the HP detection rate by 33% compared with RUT alone. Given its modest sensitivity, histology plays an important role in the diagnosis of HP infection, especially in patients taking PPIs. We recommend doing histology when RUT is negative to increase the HP detection rate.

Key words: retrospective, *Helicobacter pylori*, rapid urease test, histology, Giemsa stain

INTRODUCTION

Helicobacter pylori (HP) is a microaerophilic fastidious gram-negative bacterium involved in the pathogenesis of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma [1,2].

The diagnostic methods available for detecting HP infection include *H.pylori* stool antigen test, histology, polymerase chain reaction, rapid

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urease test (RUT), serology, and urea breath test [3]. Locally, RUT is one of the most common tests used for diagnosing HP infection. It is an invasive test that requires sampling of the gastric mucosa and provides indirect evidence of the infection by identifying the presence of the urease enzyme [4]. It is highly specific and requires a high density of bacteria. HP urease hydrolyzes urea, liberating ammonia, which produces an alkaline pH and a resultant color change of the phenolphthalein test medium. RUT is recommended initially because it is efficient, accurate and inexpensive [5]. Furthermore, RUT is extremely valuable because it gives a positive result for HP infection before the patient leaves the endoscopy unit. Its main disadvantage is that it is less accurate in patients taking proton pump inhibitors (PPI), antibiotics, or bismuth-containing compounds because these drugs reduce bacterial density and lead to false-negative results [3,5,6].

Histology remains to be the diagnostic gold standard to which other tests are compared. It has excellent sensitivity and specificity and provides additional information about the gastric mucosa [5,7]. It relies on the presence of the typical bacteria along with the inflammatory reaction for diagnosing HP infection. The routine hematoxylin and eosin (H&E) stain helps in the evaluation of the severity of inflammation along with detection of the bacteria [1]; while Giemsa stain seems to have advantage over other stains because of its simplicity and consistency in improving detection of HP [6]. Several limitations hinder routine use of histology in clinical practice including higher cost, longer turnaround time, dependence on the skills of the pathologist, and inter-observer variability. Additionally, the density of HP can vary at different sites, possibly leading to sampling error [3,6].

In a study by Yakoob et al, the sensitivity and specificity of RUT were reduced in patients taking PPI. The sensitivity and specificity of RUT with and without PPI were 43.3%, 86.4% vs 71.9% and 80%, respectively. They concluded that the exclusive use of the RUT for the diagnosis of HP cannot be recommended in patients with prior PPI use [3].

The objectives of this study were to determine the sensitivity and specificity of RUT compared with histology and to assess the detection rate of combined RUT and histology for HP infection.

MATERIALS AND METHODS

Using a retrospective design, the study was conducted in the endoscopy unit of a tertiary teaching hospital in Manila, Philippines. Purposive sampling of records was done. Preliminary screening of data was achieved by reviewing the electronic records of 3,424 in-patients and out-patients aged ≥ 18 years old from January 2017 to December 2018. Inclusion criteria were all patients who were tested for both RUT and histology at the time of the esophagogastroduodenoscopy (EGD). Baseline information such as age, gender, and symptoms were obtained as well as the corresponding endoscopic findings. Patients who underwent EGD with no determination of HP infection status by RUT and histology were excluded. The gold standard test for the diagnosis of HP infection was histology (Figure 1).

Esophagogastroduodenoscopy

An EGD was performed using Olympus® GIF HQ-190 with local xylocaine spray with or without sedation after an overnight fast of at least 6 hours. Endoscopic findings and diagnoses were made by a single gastroenterologist and at least one assisting gastroenterology fellow-in-training. At least two gastric mucosal biopsies, one from the corpus and one from the antrum, were taken both for RUT and histology.

Rapid Urease Test

Biopsy samples approximately 2–3 mm each were taken, one from the corpus and one from the antrum. These were placed on the RUT kit. The RUT kit (Lituo Biotech Company®) includes a test card that has a yellow, round indicator containing urea and a pH indicator. The production of the urease enzyme by HP results in the decomposition of urea into bicarbonate and ammonia which causes the pH to rise and the color of the indicator to change from yellow to red or pink. Positive results were read within 5 to 30 minutes. Samples that had no color change after one hour were regarded as negative [4].

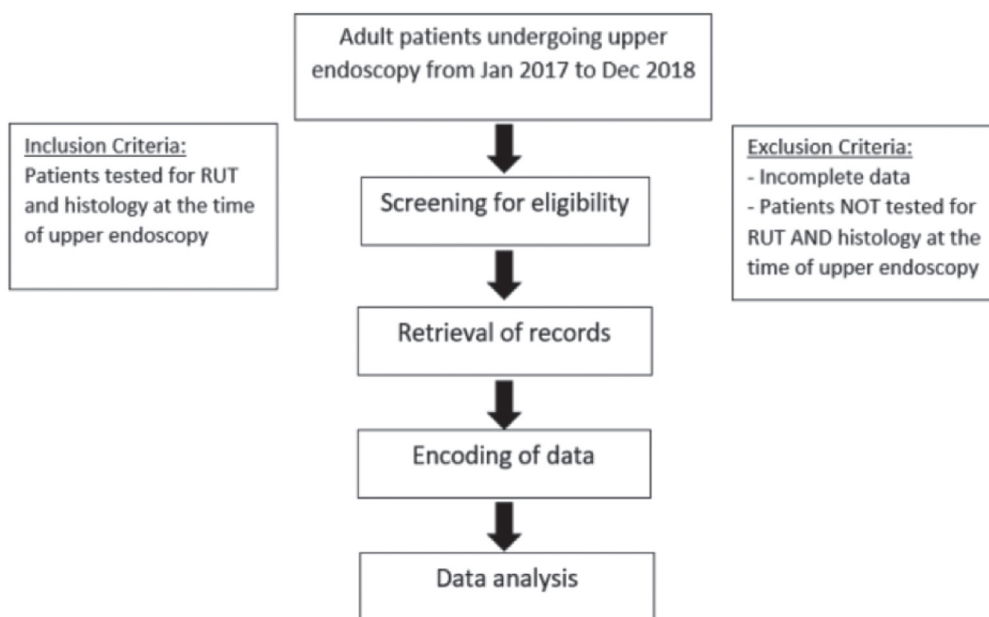


Figure 1. Study design of the combined RUT and histology for the diagnosis of HP infection

Histology

Biopsy samples approximately 2–3 mm each were taken, one from the corpus and one from the antrum were placed on a formalin bottle. A single pathologist was responsible for interpreting the histology using routine H&E and Giemsa stain.

Statistical Analysis

All data retrieved from charts and endoscopic reports were reviewed, recorded, and encoded in an electronic spreadsheet (Microsoft Excel 2016). These data were imported into Confidence Interval Calculator (2011) provided by Rob Herbert (PSG Research Workshop 2018) and IBM® SPSS® Statistics version 23 for statistical analyses. Data were summarized as frequencies and proportions. With an alpha (level of confidence) of 0.05 and 5% margin of error, 169 subjects were needed in the study to meet minimum requirements (Figure 1).

RESULTS AND DISCUSSION

A total of 192 subjects were included in the study. The mean ages for patients tested for RUT and histology were 54±17 years. Among these, 52 (27.1%) were males and 140 (72.9%) were females. Epigastric pain was the most common indication for EGD in 42.7% of patients.

Endoscopic diagnoses of patients tested for RUT and histology were as follows: 120 (62.5%)

patients had esophagitis, 44 (22.9%) had peptic ulcer disease (PUD), 120 (62.5%) had acute gastric mucosal erosions, 153 (79.9%) had chronic atrophic gastritis, and 2 (1%) had adenocarcinoma. None of the patients had normal EGD result (Table 1 and Figure 2).

Twenty-four patients (12.5%) tested positive for HP infection. Among these, 16 (8.3%) tested positive for both RUT and histology (true-positive), while 8 (4.2%) tested negative for RUT but had positive histology (false-negative). The sensitivity and specificity of RUT for the diagnosis of HP infection were 66.7 and 98.2%, respectively. While the positive and negative likelihood ratio were 37.3 and 0.34, respectively with a diagnostic odds ratio of 110 (Table 2). The addition of histologic examination to RUT increased the HP detection rate by 33% compared with RUT alone.

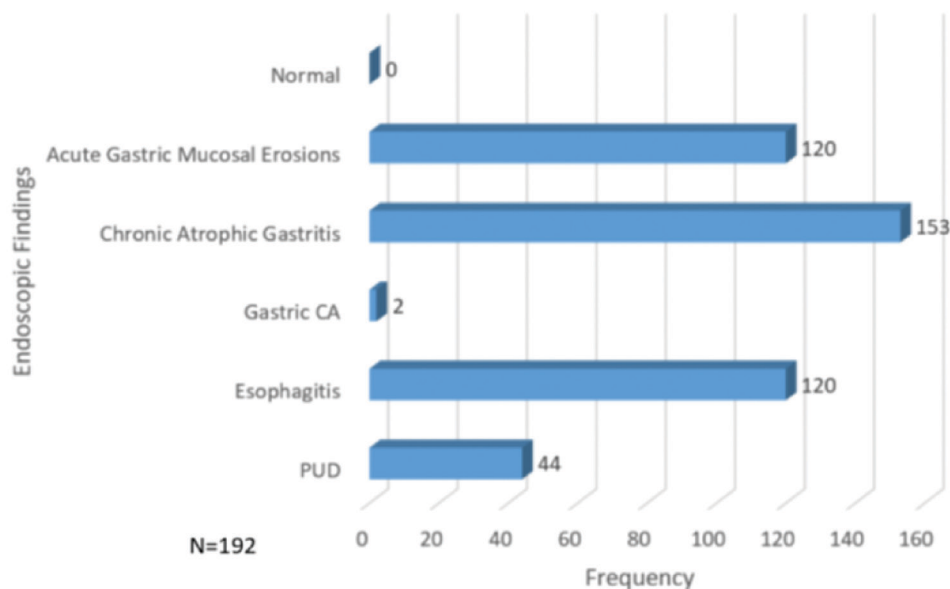
Moreover, 6 out of the 8 (75%) patients with false negative results had PPI use (Table 2). We failed to do a subgroup analysis of PPI use of all the patients included in this study due to incomplete data.

Currently, no local protocol exists in the country for the diagnosis of HP. RUT is the commonly used diagnostic test for HP because of its lower cost, and rapidity of the results and availability. However, histologic examination remains the gold standard, although this is not commonly used.

We follow the combined corpus and antrum biopsy approach when doing RUT as it is the most widely recognized worldwide [1,5]. Megraud and

Table 1. Demographic and clinical features, and endoscopic findings of patients tested using RUT and histology.

Gender	Frequency	Percentage (%)	Endoscopic Findings	Frequency	Percentage (%)
Male	52	27.1	Esophagitis		
Female	140	72.9	Yes	120	62.5
Total	192	100	No	72	37.5
Indications for EGD			PUD		
Epigastric pain	82	42.7	Yes	44	22.9
Abdominal pain	23	12	No	148	77.1
Melena	12	6.3	Acute Gastric Mucosal Erosions		
Hematemesis	9	4.7	Yes	120	62.5
Bloatedness	14	7.3	No	72	37.5
Screening	12	6.3	Chronic Atrophic Gastritis		
Surveillance	10	5.2	Yes	153	79.9
Heartburn/Regurgitation	9	4.7	No	39	20.3
Vomiting	5	2.6	Gastric Adenocarcinoma		
Dysphagia	5	2.6	Yes	2	1.0
Anemia	10	5.2	No	190	99.0
Globus	1	0.5	Normal		
Total	192	100	Yes	0	0.0
			No	192	100.0

**Figure 2.** Endoscopic findings of patients tested using RUT and histology

Lehours have recommended to take at least two biopsy specimens from the antrum and one each specimen from the anterior and posterior corpus. Because HP has a patchy distribution in the stomach, it is advisable to collect multiple biopsy specimens. More importantly, it has been observed that the corpus may be the only site which remains positive due to consumption of PPIs [1].

A common scenario in patients referred for EGD is that they have already taken PPIs, antibiotics, or bismuth-containing compounds within 2 weeks

of the procedure. To improve RUT sensitivity in such patients, stopping the potentially test-altering medication and delaying EGD for at least 2 weeks may be done [5].

This study utilized RUT combined with histologic examination, which is considered the gold standard for identifying HP infection with reported sensitivity and specificity of 95% and 98%, respectively [5]. The prevalence of HP infection in this series using RUT alone was 8.3%. When combined with histology, the prevalence of HP infection increased to 12.5%.

Table 2. Tests for diagnostic accuracy of rapid urease test versus histology

	Histology positive	Histology negative	Total
RUT positive	16	3	19
RUT negative	8	165	173
Total	24	168	192

RESULT:

Sensitivity:	0.6667	CI: 0.4671 to 0.8203
Specificity:	0.9821	CI: 0.9488 to 0.9939
Positive likelihood ratio:	37.333	CI: 11.744 to 118.683
Negative likelihood ratio:	0.339	CI: 0.193 to 0.598
Diagnostic odds ratio:	110.000	CI: 26.517 to 456.308

Thus, there was a 4.2% increase in diagnostic yield and a 33% increase in HP detection rate when using RUT combined with histology. Further studies are recommended specifically with subgroup analysis of PPI use in order to determine if this strategy is cost-effective and if it will change local and international test protocols for HP.

LIMITATIONS

The attending physician was a female gastroenterologist and a perceived risk of patient selection gender bias was anticipated due to local culture. This study was also undertaken in a single tertiary center, which may not be a general representation of patients suffering from HP infection

in the country, although the patients seen in the institution come from a variety of ethnic groups and socioeconomic backgrounds. A multi-center study may be able to include more subjects.

CONCLUSION

The HP detection rate of RUT combined with histology increased by 33% compared with RUT alone. RUT is a highly specific test for diagnosing HP infection. Given its modest sensitivity, histology plays an important role in the diagnosis of HP infection, especially in patients taking proton pump inhibitors. We recommend doing histology when RUT is negative to increase the HP detection rate.

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