Original article

Increasing detection rate of *Helicobacter pylori* infection in nonvariceal upper gastrointestinal bleeding patients by adding histology to the rapid urease test: An experience from Lampang Hospital

Charan Pankongngam

Department of Internal Medicine, Lampang Hospital

Objective To evaluate the detection rate of *H. pylori* in upper gastrointestinal bleeding (UGIB) patients using the rapid urease test (RUT) alone and in combination with histology.

Methods A prospective study of nonvariceal UGIB patients at the Internal Medicine Ward at Lampang Hospital from January to September 2016. Esophagogastroduodenoscopy (EGD) was performed and biopsy samples from the gastric body and the antrum were collected for RUT and histology to detect *H. pylori* infection. Detection rates with both strategies alone and in combination were compared using exact probability test.

Results The detection rate of *H. pylori* infection was 20.7% with RUT alone, 24.8% with histology alone, and 34.5% with a combination of histology and RUT (p =0.006).

Conclusions The detection rate of *H. pylori* infection among nonvariceal UGIB patients can be increased by adding histology to RUT, especially when RUT is negative. This strategy should be introduced universally to improve overall *H. pylori* detection rate. **Chiang Mai Medical Journal 2017;56(4):187-93.**

Keywords: upper gastrointestinal bleeding, histology, rapid urease test, *Helicobacter pylori*

Introduction

Acute upper gastrointestinal bleeding (UGIB) is a common occurrence and is considered a major emergency. Most patients who are admitted to the internal medicine ward receive endoscopic diagnosis and treatment. Lampang Hospital UGIB patients are divided into two groups: variceal and nonvariceal bleeding. Portal hypertension causes variceal bleeding from both esophageal and gastric varices as well as portal hypertensive gastropathy. The most common cause of portal hypertension is cirrhosis. The major causes of nonvariceal bleeding include peptic ulcer, gastritis, Dieulafoy's lesion, gastroesophageal laceration, and malignancy.

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that colonizes the human stomach. It was first identified in 1982 by Barry Marshall and Robin Warren, who found that it was present in individuals with chronic gastritis

Address correspondence to: Charan Pankongngam, M.D., Department of Internal Medicine, Lampang Hospital, 52000, Thailand. E-mail: pcharan1964@gmail.com

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and gastric ulcers (1). *H. pylori* is involved in the pathogenesis of atrophic gastritis, gastroduodenal ulcer, gastric cancer, MALT lymphoma, idiopathic thrombocytopenic purpura, iron deficiency anemia, and vitamin B12 deficiency (2). *H. pylori* infection also increases the risk of UGIB (3). *H. pylori* adheres to the gastric epithelium and renders the underlying mucosa more vulnerable to damage by producing enzymes and toxins (4). Eradication of *H. pylori* infection reduces the long-term rate of rebleeding and promotes healing in infected peptic ulcers (5-7).

Diagnosis of *H. pylori* infection can be made by both invasive and noninvasive methods. Noninvasive methods include the urea breath test (UBT), a serology test, and a rapid stool test for the *H. pylori* antigen. Invasive methods include endoscopic biopsy-based tests such as the rapid urease test (RUT), histology, and tissue culture (8). The RUT is an indirect test of the presence of *H. pylori* based on the presence of urease in the gastric mucosa. The urease enzyme is produced by *H. pylori*. The actual RUT results will depend on the specific gastric disease and the presence of atrophic changes or exogenous factors that can reduce the bacterial load, and thus decrease the amount of urease, producing false negative results. The sensitivity and specificity of RUT are both normally high, but with bleeding peptic ulcers the sensitivity is relatively low (9). The histology test for the presence of *H. pylori* in gastric mucosa involves staining biopsy tissues. Both methods can be performed in the same esophagogastroduodenoscopy (EGD). Early EGD is recommended for most UGIB patients because it confirms the diagnosis and allows for targeted endoscopic treatment.

Prevalence of *H. pylori* infection at Lampang Hospital detected by RUT was 50-60 percent (10, 11). Endoscopic records from 2014 indicate that the prevalence of infection has gradually decreased. The infection rate in patients with dyspepsia was 28 percent, and in those with UGIB was 16 percent.

As mentioned above, detection of *H. pylori* infection by RUT alone may be insufficient for diagnosis of *H. pylori* infection in UGIB patients (12). We designed this study specifically to evaluate the detection rate of *H. pylori* infection with RUT alone and with the addition of histology to RUT in Thai nonvariceal UGIB patients in our own practice at Lampang Hospital.

Methods

A prospective diagnostic research study was conducted from January to September 2016 with nonvariceal UGIB patients at the Department of Internal Medicine, Lampang Hospital. A study flow is shown in Figure 1.

Exclusion criteria were variceal UGIB, history of gastric surgery, hemodynamic instability, coagulopathy, or platelet count less than 50,000. All patients underwent upper EGD to investigate the cause of bleeding, and therapeutic endoscopic procedures were performed if needed. Demographic characteristics and endoscopic findings were collected.

Gastric biopsy samples were collected from two sites, the antrum and the corpus, (13), for RUT (HPOne Test®, GI Supply, Camp Hill, PA, USA; sensitivity 92.3%, specificity 100%), and histology. Two of the gastric mucosal biopsy samples, one from the corpus and one

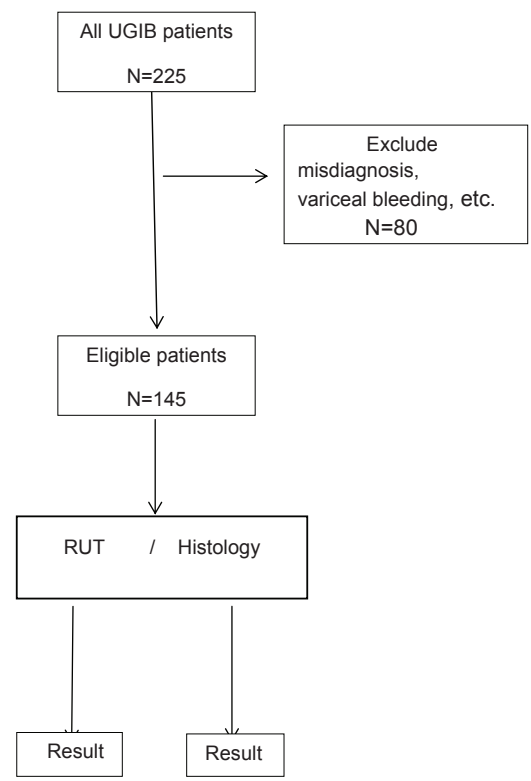

Figure 1. Study flow

from the antrum, were placed in sample wells and a reagent containing urea, a pH dye indicator, and hydrogen peroxide was placed over the specimens. The urease in *H. pylori* converts the urea to ammonia, which raises the pH and changes the color of the reagent in 60 minutes, indicating a positive RUT test.

The remaining specimens were fixed in formalin and sent to the hospital pathology department for detection of *H. pylori*. The tissues were first stained with hematoxylin and eosin. If, in the opinion of the pathologist, the results were inconclusive, the specimens would be analyzed using the Warthin-Starry method (14, 15).

The study protocol was approved by the Ethics Committee of Lampang Hospital. The patients or their representatives signed an informed consent form prior to participation.

Sample size, and statistical analysis

Sample size was calculated on the basis of the 16% detection rate of *H. pylori* infection in UGIB patients found by RUT at Lampang Hospital in 2014. The study hypothesis was that adding histology to RUT would increase the detection rate by 50 percent over the base line RUT (an overall increase of 16 to 24 percent). Using one sample comparison of proportion to hypothesized value, given p <0.05 and 80% statistical power, the minimum required sample size was 145.

Descriptive data are presented as number, percent, mean, and standard deviation. The detection rates were compared using a probability test.

Results

A total of 145 nonvariceal UGIB patients at the Internal Medicine ward at Lampang Hospital underwent EGD between January and September 2016. Of those, 102 patients (70.3%) were male. The mean age was 64.5±16.5 years (range 17-98 years). Melena was the most common presentation. Hematemesis was frequently observed, but both coffee grounds vomits and hematochezia were not. Thirty eight percent of patients had abdominal pain. More than half the patients had a history of NSAIDs use. Treatment with intravenous proton pump inhibitors is a standard treatment and only four patients had not received PPI before EGD, but only six patients had used antibiotics and none had used bismuth. Omeprazole was the most frequently used PPI; however, only four patients used a pantoprazole intravenous drip. The average time from admission to EGD was 28.6±18.9 hours (range 0-72) (Table 1).

The three most common etiologies of nonvariceal UGIB were gastric ulcer (GU) at 47.6%, duodenal ulcer (DU) at 38.6%, and gastritis at 29.7%. Adenocarcinoma of the stomach was diagnosed in two patients (1.4%). An additional AFB stain was positive in a specimen from an elderly woman who had GU. Tuberculosis of the stomach, a very rare condition, was diagnosed. At the time of the EGD session, bleeding in most patients had stopped and only 9.7 percent had blood in the stomach. Therapeutic endoscopic procedures were performed in 11 patients (Table 2).

RUT detected *H. pylori* infection in 30 patients (20.7%), while histology detected infection in 36 (24.8%) (p =0.484). Among patients with a negative RUT, 20 were later determined to have *H. pylori* infection by histology. Thus, detection of *H. pylori* by a combination of histology and RUT brought the total of patients diagnosed as harboring *H. pylori* to 50 (34.5%). All 36 histology positive patients were also RUT positive. There were, however, 14 patients (9.7%) who were RUT positive for whom *H. pylori* could not be found histologically (Table 3).

Table 1. Demographic data (n=145)

Characteristics	N (%)
Sex (male)	102 (70.3%)
Age (years); mean±SD, (range)	64.5±16.5, (17-98)
Hematemesis	65 (44.8%)
Coffee grounds vomits	17 (11.7%)
Hematochezia	6 (4.1%)
Melena	100 (69.0%)
Abdominal pain	56 (38.6%)
NSAIDs use (preceding 4 wks)	87 (60.0%)
Antibiotics use (preceding 2 wks)	6 (4.1%)
Oral PPI use (preceding 2 wks)	11 (7.6%)
Bismuth use (preceding 4 wks)	0 (0.0%)
IV PPI use	141 (97.2%)
Omeprazole	139 (95.9%)
Pantoprazole	4 (2.8%)
Time to endoscopy after admission (hours); mean±SD, (range)	28.6±18.9, (0-72)

Table 2. Endoscopic findings, *H. pylori* test results, and treatment

Findings and treatment	N (%)
Endoscopic findings	
Normal	0 (0.0)
GU	60 (47.6)
DU	56 (38.6)
Gastritis	43 (29.7)
Gastroesophageal laceration	8 (5.5)
Dieulafoy's lesion	0 (0.0)
Esophageal lesion	9 (6.2)
GERD	9 (6.2)
Malignancy	2 (1.4)
Blood in stomach	14 (9.7)
Coffee grounds in stomach	3 (2.1)
Fresh or clotted blood in stomach	11 (7.6)
H. pylori test results and diagnosis	
RUT positive	30 (20.7)
Histology positive	36 (24.8)
H. pylori infection	50 (34.5)
Post-diagnosis treatment	
Endoscopic treatment	11 (7.6)
Adrenaline injection	5 (3.5)
Heat probe coagulation	4 (2.8)
Hemoclipping	5 (3.5)
Complications from treatment	0 (0.0)

We used a probability test to compare the detection rates of *H. pylori* infection in nonvariceal UGIB patients using RUT alone and using a combination of RUT plus histology, and found the difference was statistically significant (p =0.006).

The *H. pylori* infection rate was 34.78% in GU patients and 33.93% in DU patients; that difference was not statistically significant (p =1.000).

In cases where blood was present in the stomach, RUT detected *H. pylori* infection in 3 of 14 patients, histology detected infection in 1 of 14, and the combined tests detected infection in 3 of 14. The sensitivity of the RUT, histology, and combined tests were 21.4%, 7.1% and 21.4%, respectively. In the absence of blood, RUT detected *H. pylori* infection in 27 of 131 patients, histology detected it in 35 of 131, and the combined tests detected it in 47 of 131. The sensitivity of the tests were 20.6%, 26.7% and 35.9%, respectively.

Discussion

H. pylori is involved in the pathogenesis of atrophic gastritis, gastroduodenal ulcer, gastric cancer, MALT lymphoma, idiopathic thrombocytopenic purpura, iron deficiency anemia, and vitamin B12 deficiency. *H. pylori* infection increases the risk of UGIB. Eradication of *H. pylori* infection reduces the long-term rate of rebleeding and promotes healing in infected peptic ulcers. EGD was performed in UGIB patients in this study to identify the etiology, to detect *H. pylori* infection, and to perform endoscopic therapy.

Gastric biopsy specimens were collected and tested using RUT, the results of which can be read within 60 minutes. Other biopsy specimens were sent to the pathology department, where reports might require up to 2 weeks to complete.

The detection rate of *H. pylori* infection in this study using RUT alone was low (20.7%), but increased markedly with the addition of a histology test (34.5%, p =0.006). Histology alone cannot replace RUT in the detection of *H. pylori* infection and the rate of detection using either RUT or histology alone was not significantly different (p =0.484).

RUT is a test for the presence of the urease enzyme. A positive RUT result requires a minimum of 100,000 bacteria to be present in the

Table 3. Detection of *H. pylori* infection

	Cases	RUT	Histology	*H. pylori* detection	*p-value**
RUT plus histology	16	16	16	16	
RUT alone	14	14	14	14	
Histology alone	20	20	20	20	
Negative both tests	95	95	95	-	
Infected case ratio	-	-	-	50/145	
Detection rate (%)	-	-	-	34.5	0.006

biopsy sample. Many factors can interfere with the detection of *H. pylori* infection using RUT. Results depend on the type of gastric disease and the presence or absence of atrophic changes and other exogenous factors that can reduce the bacterial load and, as a consequence, the quantity of urease enzyme, thus producing false negative results. In addition, the use of antibiotics, bismuth-containing compounds, or proton pump inhibitors reduces the bacterial density and may result in false-negative results (16,17). PPIs have been shown to have a bactericidal effect on the organism prior to the test. On the other hand, false positive results can occur if other urease-containing organisms are present in sufficient quantity or if the specimen is allowed to contact the media for a prolonged period. Most of the patients in this study had received intravenous PPI and six patients had used antibiotics, but none had used bismuth. It has been postulated that blood in the stomach leads to decreased sensitivity of RUT, possibly related to the presence of albumin (18). Another postulate is that blood has a pH buffering effect because more alkaline settings are known to be associated with more false-negative results (19). In this study only 9.7% of the patients had blood in the stomach. In the presence of blood the sensitivity of RUT alone, histology alone, and the combined tests were 21.4%, 7.1% and 21.4%, respectively. This may not represent the true sensitivity due to small number of patients. In the absence of blood, the sensitivities of the tests were 20.6%, 26.7% and 35.9%, respectively.

The prevalence of *H. pylori* infection in UGIB patients detected by RUT at Chulalongkorn Hospital was 27 percent, but that included variceal UGIB patients (20).

The rapid urease test is a popular diagnostic test because it is a quick, cheap and simple way to detect the presence of urease in or on the gastric mucosa. The sensitivity and specificity of the test are generally high. But under conditions where the RUT yield may decrease, e.g., in the presence of blood or in patients who are on PPI treatment, other methods to detect *H. pylori* infection should be considered. Our study clearly showed that RUT detection of *H. pylori* infection under such conditions was abnormally low and that adding the histology test increased the positive detection rate.

In general, the cost of histopathology is much higher than RUT, especially if the specimen needs a special stain such as Warthin-Starry. In addition, histology also delays reporting of results as a specialist is needed to evaluate the tissue.

We suggest that to improve the detection rate of *H. pylori* infection and to minimize the cost, biopsies should be taken for both RUT as well as for histology from normal-appearing mucosa from the corpus and the antrum, and then if the RUT results are positive, the histology specimens can be discarded without further examination. Other diagnostic tests including serology, histology, urea breath test, rapid urease test, stool antigen, and culture should be deferred, as they demonstrate a low negative predictive value in the setting of acute UGIB (12). If both the RUT and histology are negative, there may be a role for other non-invasive diagnostic tests at certain period after completion of UGIB treatment.

Conclusions

The efficacy of detection of *H. pylori* infection in nonvariceal UGIB using RUT alone is relatively low, but the addition of histology can markedly increase the detection rate. Gastric biopsy specimens should be collected from all UGIB patients who undergo EGD for RUT as well as for histology. If the RUT is positive, a diagnosis of *H. pylori* infection can be made. If the RUT is negative, then it is appropriate to wait for histology results before making a firm diagnosis.

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This study provided patients the standard of care

service. Statistical consultation was provided on an complimentary basis.

Conflicts of interest

The author has no conflicts of interest to report.

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การตรวจทางพยาธิวิทยาช่วยเพิ่มอัตราการตรวจพบเชื้อเฮลิโคแบคเตอร์ ไพลอรีในผู้ป่วยเลือดออกในทางเดินอาหารส่วนต้นจากที่ตรวจด้วยการหายูรีเอสเอนไซม์แบบเร็วเท่านั้น: ประสบการณ์จากโรงพยาบาลลำปาง

จรัล ปันกองงาม
กลุ่มงานอายุรกรรม โรงพยาบาลลำปาง

วัตถุประสงค์ เพื่อศึกษาอัตราการตรวจพบการติดเชื้อเฮลิโคแบคเตอร์ ไพลอรีในผู้ป่วยเลือดออกในทางเดินอาหารส่วนต้นที่ไม่ได้เกิดจากหลอดเลือดทางเดินอาหารโป่งพอง จากวิธีตรวจด้วยการหายูรีเอสเอนไซม์แบบเร็วเท่านั้น และวิธีส่งตรวจทางพยาธิวิทยาเพิ่มเติม

วิธีการ ศึกษาไปข้างหน้าในผู้ป่วยเลือดออกจากทางเดินอาหารส่วนต้นที่ไม่ได้เกิดจากหลอดเลือดทางเดินอาหารโป่งพอง ที่รับไว้รักษาเป็นผู้ป่วยในแผนกอายุรกรรมโรงพยาบาลลำปางตั้งแต่เดือนมกราคมถึงกันยายน 2559 โดยส่องกล้องตรวจทางเดินอาหารส่วนต้นและตัดชิ้นเนื้อที่ตัวกระเพาะและส่วนปลายเพื่อตรวจหาการติดเชื้อเฮลิโคแบคเตอร์ ไพลอรีโดยวิธีการตรวจหายูรีเอสเอนไซม์แบบเร็วและวิธีส่งตรวจทางพยาธิวิทยา เปรียบเทียบอัตราการตรวจพบการติดเชื้อจากทั้งสองวิธี และเปรียบเทียบวิธีการตรวจหายูรีเอสเอนไซม์แบบเร็วเท่านั้นกับวิธีส่งตรวจทางพยาธิวิทยาเพิ่มเติมจากวิธีการตรวจหายูรีเอสเอนไซม์แบบเร็ว โดยใช้ exact probability test.

ผลการศึกษา อัตราการตรวจพบการติดเชื้อเฮลิโคแบคเตอร์ ไพลอรีโดยวิธีการตรวจหายูรีเอสเอนไซม์แบบเร็วเท่านั้นร้อยละ 20.7 วิธีส่งตรวจทางพยาธิวิทยาร้อยละ 24.8 วิธีส่งตรวจทางพยาธิวิทยาเพิ่มเติมจากวิธีการตรวจหายูรีเอสเอนไซม์แบบเร็วร้อยละ 34.5 (p =0.006)

สรุป สามารถเพิ่มอัตราการตรวจพบการติดเชื้อเฮลิโคแบคเตอร์ ไพลอรีในผู้ป่วยเลือดออกในทางเดินอาหารส่วนต้นที่ไม่ได้เกิดจากหลอดเลือดทางเดินอาหารโป่งพองจากที่ตรวจด้วยการหายูรีเอสเอนไซม์แบบเร็วเท่านั้น โดยการส่งตรวจตรวจทางพยาธิวิทยาเพิ่มเติมโดยเฉพาะเมื่อผลการตรวจด้วยการหายูรีเอสเอนไซม์แบบเร็วเป็นลบ กลยุทธนี้ควรใช้โดยแพร่หลายเพื่อให้วินิจฉัยการติดเชื้อได้ดีขึ้น **เชียงใหม่เวชสาร 2560;56(4):187-93.**

คำสำคัญ: upper gastrointestinal bleeding, histology, rapid urease test, *Helicobacter pylori*