Original Article

Gastric Juice PCR for The Diagnosis of *Helicobacter pylori* Infection in Patients with Upper Gastrointestinal Bleeding

Chandrasakha S
Hansombun P
Sirinawasatien A

ABSTRACT

Aims: To determine the efficacy of gastric juice polymerase chain reaction (PCR) for the detection of *H. pylori* infection in comparison with histology, rapid urease test and culture in patients with upper gastrointestinal bleeding.

Method: Sixty-four patients with upper gastrointestinal bleeding were enrolled. At endoscopy, gastric juice for PCR of *H. pylori* and gastric biopsies for rapid urease test (RUT), histology and culture were collected.

Results: There were 53.1% of patients presenting with melena and 26.6% with hematemesis. Endoscopic findings included lesions in the stomach (58 patients) and in the duodenum (13 patients). *H. pylori* infection was found in 43.8%. The sensitivity of gastric juice PCR was significantly higher than histology (92.9% vs. 25%, $p<0.001$) but equal to that of rapid urease test (92.9%) and culture (96.4%) ($p<0.001$). Further analysis showed non-significant difference in the sensitivities of rapid urease test, histology, culture, and gastric juice PCR between patients with or without blood in the stomach.

Conclusions: Gastric juice PCR is highly sensitive for detecting *H. pylori* in patients with upper gastrointestinal bleeding. The sensitivity of gastric juice PCR is similar to rapid urease test, histology, and culture moreover this method is non-invasive and non-biopsy-based.

Key words : *H. pylori* infection, gastric juice PCR, rapid urease test

[Thai J Gastroenterol 2014; 15(3):128-134.]

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral gram-negative microaerophilic bacterium which was first described by Warren and Marshall[1-4]. It is a causative agent of chronic gastritis and gastric and duodenal ulceration, as well as an independent risk factor for gastric carcinoma and MALToma[5-8]. The prevalence of *H. pylori* is approximately 70% in patients with bleeding peptic ulcers[9-10]. Bleeding is common and is a serious complication of gastric ulcer. Numerous studies have shown that eradicating *H. pylori* infection can reduce the incidence of re-bleeding[11-13]. *H. pylori* infection is diagnosed by an invasive esophagogastroduodenoscopy (EGD), or non-invasively in which EGD

Division of Gastroenterology and Hepatology, Department of Medicine, Rajavithi Hospital, Bangkok, Thailand.
Address for Correspondence: *Apichet Sirinawasatien, M.D., Division of Gastroenterology and Hepatology, Department of Medicine, Rajavithi Hospital, Bangkok, Thailand.*

is not needed. The invasive diagnostic tests include bacterial culture, histology and rapid urease test. The non-invasive tests are such as serology, stool antigen test, and urea breath test. In our practice, rapid urease test and histology are the two common methods to confirm the presence of *H. pylori* infection. However, many studies have shown that in the setting of ulcer bleeding, rapid urease test and histology lack the sensitivity. Gastric juice PCR is a noninvasive diagnostic method for *H. pylori* infection. Gastric juice PCR can diagnose *H. pylori* infection when performed in non-bleeding situation and is more accurate than histology. However, there are no studies in patients with upper gastrointestinal bleeding. We therefore conduct this study to evaluate the sensitivity, specificity and accuracy of gastric juice PCR assay for detecting *H. pylori* infection in patients with upper gastrointestinal bleeding.

Material and method

Patients: Sixty-four consecutive patients with hematemesis, melena,or both, who underwent EGD at Rajavithi Hospital, were enrolled in the study. Exclusion criteria were age <15 or > 80 years; underlying liver cirrhosis; history of coagulopathy or other disorders contraindicated for EGD or biopsy sampling; and previous history of *H. pylori* therapy. Data collected age, sex, medical history, underlying disease, drug history, alcohol consumption, presenting symptom(s), Rockall score, Blatchford score, gastroduodenal lesion(s) and presence or absence of blood in the stomach were recorded. An informed consent was obtained from each subject. This study was approved by the Human Medical Ethics Committee of Rajavithi Hospital, Bangkok, Thailand.

Endoscopy and sample collection

During endoscopy, 5 mL of gastric juice was aspirated via the endoscope using a sterile tube that was passed through the suction channel. The gastric fluid was collected in a disposable sterile syringe. Biopsy forceps were sterilized by autoclaving to eliminate cross contamination of the endoscopic equipment. Gastric biopsy specimens were taken for rapid urease test (1 from the gastric body and 1 from the antrum), histology (1 from the body and 1 from the antrum), culture (1 from the body and 1 from the antrum). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, positive and negative likelihood ratio for gastric juice *H. pylori* PCR were compared against histology, rapid urease test, and *H. pylori* to establish the efficacy of the diagnostic approach.

Rapid urease test

Gastric biopsy specimens from the antrum and the gastric body were tested by Pronto® dye test (Medical Instrument Corp, Solothum, Swizerland), which was read in 60 minutes after sampling. Color change from yellow to pink was considered to indicate a positive test.

Histological examination

Gastric biopsy specimens from the antrum and the gastric body for histopathology were stained with hematoxylin and eosin for the detection of *H. pylori.* The degree of gastritis was scored using Sydney system[14]. The specimens were interpreted by a histopathologist who was blinded to the patient status and the results of other laboratory tests.

H. pylori culture

Gastric biopsy specimens from the antrum and the gastric body for culture were transferred in brain-heart infusion for examination. A positive culture was defined as bacterial growth within 7 days. The organisms were identified as *H. pylori* by gram-stain, colony morphology and positive oxidase, catalase and urease reactions.

Gold standard diagnosis

A patient was classified as being *H. pylori* positive on the basis of a positive culture or a positive rapid urease test plus histopathology.

Gastric juice PCR

5 mL of gastric juice was buffered to neutral pH with 5 mL of Tris [0.67 mol/L, pH 7.4]. The sample was centrifuged at 10,000*g for 20 mins. The supernatant was then separated, and the pellet component combined with sterile distilled 100 µL of lysis buffer [100 MMol/L NaCl, 10 MMol/L Tris-HCl pH8.0], 20 hrs chloroform extraction and ethanol precipitation, in accordance with the previous study[10].

PCR amplification

DNA extraction was performed using an automated DNA extraction [Magna pure Compact Nucleic

and Isolation Kit-Roche], after which the DNA was eluted in 200 μL of nuclease-free water and stored at -200c for subsequent real time polymerase chain reaction [RT-PCR] analysis using Hybridization probe and primer of [Tibmol, Germany]. The forward primer HP23S1 [5'-GGA GCT GTC TCA ACC AGA GAT TC-3'] and reversed primer HP23S2 [5'-CGC ATG ATA TTC CC [AG] TTA GCA G-3'] being the specific sequence for 23SrRNA gene of *H. pylori* and the hybridization probes HP23S3[5'-GGA GCT GTC TCA ACC AGA GA [Red640] TTC-3'] and HP23S4 [5'-GGA ATT TTC ACC TCC ACT ACA ATT TCA CTG[Fluo]-3'] were used for 23SrRNA gene of *H. pylori* in accordance with the previous study[10].

Statistical analysis

Statistical analysis was performed using SPSS system. The descriptive analysis was mode for demographic and clinical features. Results were presented as ± SD for quantitative variables and number (percentage) for qualitative variables. Sensitivity, specificity, accuracy, predictive values of positive and negative results were calculated in accordance with standard methods. χ^2 test and 95%CI were used to compare the sensitivity, specificity and accuracy of different diagnostic methods. $p<0.05$ was considered statistically significant.

RESULTS

Sixty-four patients were included in this study (Table 1) presented the demographic data of patients [41 males, 23 females, mean age 59 years, history of NSAIDS (21.9%), and aspirin use (48.4%)]. EGD showed lesion(s) in the stomach (79.7%), the duodenum (9.4%) as shown in Table 2. Based on the gold standard for diagnosis, 43.8% were *H. pylori* positive and 56.2% were H. pylori negative. *H. pylori* positivity was documented in 62.5% of all rapid urease tests, 65.6% in gastric juice PCR, and 10.9% in histology (Table 3). Table 4 presents the sensitivity, specificity and accuracy of various tests in diagnosing *H. pylori* infection. The sensitivities of rapid urease test, histology, culture, and gastric juice PCR were 92.9%, 25%, 96.4%, 92.9% respectively. For patients with intragastric blood at EGD, gastric juice PCR was positive in 60%, versus 70% in patients without intragastric blood at EGD (p=0.373).

The patients were divided into 2 groups according to the presence or absence of blood in the stomach to determine the relationships between intragastric blood and sensitivities of biopsy-based tests for *H. pylori* infection. The sensitivities of rapid urease test, histology, culture and gastric PCR were 92.9%, 42.9%, 92.9%, 92.9% in patients with intragastric blood, and 92.9%, 7.1%, 100%, 92.9% in those without intragastric blood, respectively. There were no signifi-

Table 1. Baseline characteristics of patients with upper gastrointestinal bleeding.

Characteristics	Total (n = 64)	
	Number	Percent
Age		
Mean±S.D.	59.61±15.80	
Median (min-max)	61 (18 - 85)	
Gender		
Male	41	64.1%
Female	23	35.9%
Underlying diseases		
Diabetes mellitus	16	25.0%
Hypertension	25	39.1%
CAD	7	10.9%
Dyslipidemia	11	17.2%
Chronic kidney disease	12	18.6%
HT	1	1.6%
SLE	1	1.6%
Stroke	6	9.4%
History of drugs		
Aspirin	31	48.4%
NSAIDS	14	21.9%
Symptom to EGD		
Melena	34	53.1%
Hematemesis	17	26.6%
Melena and Hematemesis	13	20.3%
Alcohol drinking	20	31.2%
History herbal used	8	12.5%
Amount of PRC transfusion (units) (n=44)		
Mean±S.D.	2.50 ± 1.41	
Median (min-max)	2 (1 - 8)	
Rockall score		
Mean±S.D.	2.52 ± 1.86	
Median (min-max)	2 (0-8)	
Blatchford score		
Mean±S.D.	11.23 ± 3.82	
Median (min-max)	3.82 (3 - 19)	

Table 2. Endoscopic findings.

EGD	Total (n = 64)	
	Number	Percent
Site of lesion		
Stomach	51	79.7%
Duodenum	6	9.4%
Stomach and Duodenum	7	10.9%
Stomach (n=58)		
Gastric ulcer	30	51.7%
-Clean base ulcer	26	86.3
-Non bleeding visible vessel	4	13.7
Erosive gastritis	15	25.8%
Hemorrhagic gastritis	8	13.8%
Non-erosive gastritis	2	3.4%
Malignancy	2	3.4%
Mallory Weiss tear	2	3.4%
Duodenum (n=13)		
Duodenal ulcer	13	100%

cant differences in the sensitivities of rapid urease test, histology, and gastric PCR between the patients with and without blood in the stomach as shown in Table 7 and Table 8.

Discussion

H. pylori infection has been associated with gastric disorders such as chronic gastritis, duodenal ulcer, gastric ulcer, and MALT lymphoma[1]. *H. pylori* status in a patient with upper gastrointestinal bleeding is of importance. The true prevalence of *H. pylori* remains controversial at about 70-90%. Many studies have noted that biopsy-based tests including rapid urease test, histology and culture all have low sensitivity to detect *H. pylori*[16-18]. In a previous study, gastric juice PCR which is a non-invasive test for the diagnosis *H. pylori* infection had a greater sensitivity than histology[15]. In this study, we also noted that the sensitivity of rapid urease test, histology and culture were 92.9%,

Table 3. Results of *H. pylori* testing by rapid urease test, histology, culture, gastric juice PCR.

	Positive		Negative	
	Number	Percent	Number	Percent
Rapid urease test	40	62.5%	24	37.5%
Histology	7	10.9%	57	89.1%
Culture	27	42.2%	37	57.8%
Culture or (Rapid urease test plus Histology)	28	43.8%	36	56.2%
Gastric juice PCR	42	65.6%	22	34.4%

Table 4. Comparison of sensitivity, specificity and accuracy of various tests in diagnosing *H. pylori* infection.

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	LR positive	LR negative
Rapid urease test	92.9 (75.0-98.8)	61.1 (43.5-76.4)	65 (48.3-78.9)	91.7 (71.5-98.5)	75.0	2.3 (1.57-3.64)	0.11 (0.03-0.46)
Histology	25.0 (11.4-45.2)	100 (88 -100)	100 (56.1-100)	63.2 (49.3-75.2)	67.2	-	0.75 (0.61-0.93)
Culture	96.4 (79.7-99.8)	100 (88-100)	100 (84.5-100)	97.3 (84.2-99.9)	98.4	-	0.04 (0.01-0.24)
Gastric juice PCR	92.9 (74.0-98.8)	55.6 (38.3-71.7)	61.9 (45.7-76.0)	90.9 (69.4-98.4)	71.9	2.09 (1.43-3.05)	0.13 (0.03-0.51)

Table 5. Comparison of gastric juice PCR, histology and rapid urease test using positive culture or positive rapid urease test plus histology as a gold standard.

	Gold Standard				*p*-value
	Positive (n=28)		Negative (n=36)		
	Number	Percent	Number	Percent	
Rapid urease test					< 0.001
Positive	26	92.9%	14	38.9%	
Negative	2	7.1%	22	61.1%	
Histology					0.002
Positive	7	25.0%	0	0%	
Negative	21	75.0%	36	100%	
Culture					< 0.001
Positive	27	96.4%	0	0%	
Negative	1	3.6%	36	100%	
Gastric juice PCR					< 0.001
Positive	26	92.9%	16	44.4%	
Negative	2	7.1%	20	55.6%	

Table 6. Effect of intra-gastric blood on sensitivity of various tests in diagnosing *H. pylori* infection.

	Intra-gastric blood				*p*-value
	Presence (n=30)		Absence (n=34)		
	Number	Percent	Number	Percent	
Rapid urease test					0.244
Positive	21	70.00%	19	55.90%	
Negative	9	30.00%	15	44.10%	
Histology					0.044
Positive	6	20.00%	1	2.90%	
Negative	24	80.00%	33	97.10%	
Culture					0.862
Positive	13	43.30%	14	41.20%	
Negative	17	56.70%	20	58.80%	
Gastric juice PCR					0.373
Positive	18	60.00%	24	70.60%	
Negative	12	40.00%	10	29.40%	

25%, 96.4%, respectively, while gastric juice PCR reached a sensitivity of 92.9%.

In our study, the sensitivities of rapid urease test, histology, culture and gastric juice PCR were 92.9%, 42.9%, 92.9% and 92.9% in patients with intra-gastric blood, and 92.9%, 7.1%, 100% and 92.9% in patients without intra-gastric blood.

In previous studies, the sensitivities of rapid urease test, histology, culture, and gastric juice PCR were reduced in the presence intra-gastric blood. These data imply that the presence of lower diagnostic yields. An in vitro study also showed that sheep's blood inhibited the growth of *H. pylori* in broth medias[19], but no difference was noted between absence or presence of

Table 7. Comparison of sensitivity, specificity and accuracy of various tests in diagnosing *H. pylori* infection when presence of intra-gastric blood.

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	LR positive	LR negative
Rapid urease test	92.9 (64.2-99.6)	50 (25.5-74.5)	61.9 (38.7-81.0)	88.9 (50.7-99.4)	70.0	1.86 (1.11-3.10)	0.14 (0.02-1.07)
Histology	42.9 (18.8-70.4)	100	100	66.7 (44.7-83.6)	73.3	-	0.57 (0.36-0.90)
Culture	92.9 (64.2-99.6)	100	100	94.1 (69.2-99.7)	96.7	-	0.07 (0.01-0.47)
Gastric juice PCR	92.9 (64.2-99.6)	68.8 (41.5-87.9)	72.2 (46.4-89.3)	91.7 (59.8-99.6)	80.0	2.97 (1.42-6.24)	0.10 (0.01-0.73)

Table 8. Comparison of sensitivity, specificity and accuracy of various tests in diagnosing *H. pylori* infection when absence of intra-gastric blood.

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	LR positive	LR negative
Rapid urease test	92.9 (64.2-99.6)	70 (45.7-87.2)	68.4 (43.5-86.4)	93.3 (66.0-99.7)	79.4	3.09 (1.56-6.14)	0.10 (0.01-0.70)
Histology	7.1 (0.4-35.8)	100	100	60.6 (42.2-76.6)	61.8	-	0.92 (0.80-1.07)
Culture	100	100	100	100	100	-	-
PCR	92.9 (64.2-99.6)	45 (23.8-67.9)	54.2 (33.2-73.8)	90 (54.1-99.5)	64.7	1.69 (1.11-2.58)	0.16 (0.02-1.18)

blood in the stomach. Further investigation is needed to clarify this observation.

Ours is the first study to detect *H. pylori* by gastric juice PCR in patients with upper gastrointestinal bleeding. The use of gastric juice PCR can be recommended to exclude *H. pylori* infection in upper gastrointestinal bleeding patients who cannot be reliably tested by biopsy-based tests such as rapid urease test, histology or culture.

Results from this study should be confirmed in a larger group of patients. Limitation of this study found the low sensitivity of classic methods in these patients make it difficult to evaluate the new method such as PCR. Therefore, it may be that results identified as false positive but rather that the results obtained with classic methods were false negatives. In this study, sensitivity of histology was lower than other method due to only stain with hematoxylin and eosin for the detection of *H. pylori.*

In conclusion, gastric juice PCR which is non-invasive, non-biopsy based test is highly sensitive for determining *H. pylori* status in patients with upper gastrointestinal bleeding. However, no significant difference was noted regarding the sensitivity of the test in the presence or in the absence of intra-gastric blood.

REFERENCES

1. Blaser MJ. Ecology of Helicobacter pylori in the human stomach. J Clin Invest 1997;100:759-62.
2. Blaser MJ, Perez-Perez GI, Kleanthous H, *et al.* Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995;55:2111-5.
3. Warren JR. Unidentified curved bacilli on gastric epithelium in active chronic gastritis [letter]. Lancet 1983;1:1273.
4. Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis [letter]. Lancet 1983;1:1273-5.
5. Fiocca R, Luinetti 0, Villani L, *et al.* High incidence of Helicobacter pylori colonization in early gastric cancer and

the possible relationship to carcinogenesis. Eur J Gastroenterol Hepatol 1993;5(suppl2):2-8.
6. Hansson LE, Engstrand L, Nyren 0, *et al.* Helicobacter pylori infection: Independent risk indicator of gastric adenocarcinoma. Gastroenterology 1993;105(4):1098-103.
7. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, *et al*. Helicobacter pylori associated gastritis and primary B-cell gastric lymphoma. Lancet 1991; 338(8776):1175-6.
8. Calvert R, Randerson J, Evans P, *et al.* Genetic abnormalities during transition from Helicobacter-pylori-associated gastritis to low-grade MALToma. Lancet 1995;345:26-7.
9. Sirinthornpunya S. Prevalence of Helicobacter pylori infection in patients with peptic disease. J Med Assoc Thai 2012 Mar;95 Suppl 3:S22-7
10. Hansomburana P, Anantapanpong S, Sirinthornpunya S, *et al.* Prevalence of singlenucleotidemutation in clarithromycin resistant gene of Helicobacter pylori: a 32-months prospective study by using hybridization real time polymerase chain reaction. J Med Assoc Thai 2012 Mar;95 Suppl 3:S28-35.
11. Rokkas T, Karameris A, Mavrogeorgis A, *et al.* N. Eradication of Helicobacter pylori reduces the possibility of rebleeding in peptic ulcer disease. Gastrointest Endosc 1995; 41: 1-4.
12. Graham DY, Hepps KS, Ramirez FC, *et al.* Treatment of Helicobacter pylori reduces the rate of rebleeding in peptic ulcer disease. Scand J Gastroenterol 1993; 28: 939-42.
13. Labenz J, Borsch G. Role of Helicobacter pylori eradication in the prevention of peptic ulcer bleeding relapse. Digestion 1994;55(1):19-23.
14. Price AB. The Sydney System: histological division. J Gastroenterol Hepatol 1991; 6: 209-222
15. Yakoob J, Rasool S, Abbas Z, *et al.* gastric juice for the diagnosis of H pylori infection in patients on proton pump inhibitors. World J Gastroenterol 2008;14(10):1539-43.
16. Lee JM, Breslin NP, Fallon C, *et al.* Rapid urease test lack sensitivity in Helicobacter pylori diagnosis when peptic ulcer disease presents with bleeding. Am J Gastroenterol 2000;95: 1166-70.
17. Tu TC, Lee CL, Wu CH, *et al.* Comparison of invasive and noninvasive tests for detecting Helicobacter pylori infection in bleeding peptic ulcers. Gastrointest Endosc 1999;49:302-6.
18. Colin R, Czernichow P, Baty V, *et al.* Low sensitivity of invasive tests for the detection of Helicobacter pylori infection in patients with bleeding ulcer. Gastroenterol Clin Biol 2000; 24:31-5.
19. Coudron PE, Stratton CW. Factors affecting growth and susceptibility testing of Helicobacter pylori in liquid media. J Clin Microbiol 1995;33:1028-30.