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• BRIEF REPORTS •

Mucosal polymerase chain reaction for diagnosing *Helicobacter pylori* infection in patients with bleeding peptic ulcers

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Abstract

AIM: *Helicobacter pylori* (*H pylori*) has been linked to chronic gastritis, peptic ulcers, gastric cancer and MALT-lymphoma. Conventional invasive tests are less sensitive than non-invasive tests in diagnosing *H pylori* infection in patients with bleeding peptic ulcers. Polymerase chain reaction is a sensitive and accurate method for diagnosing *H pylori* infection. The aim of this study was to evaluate the diagnostic role of mucosal polymerase chain reaction for *H pylori* infection in patients with bleeding peptic ulcers.

METHODS: In patients with bleeding, non-bleeding peptic ulcers and chronic gastritis, we checked rapid urease test, histology, bacterial culture and mucosal polymerase chain reaction for detecting *H pylori* infection. Positive *H pylori* infection was defined as positive culture or both a positive histology and a positive rapid urease test. For mucosal polymerase chain reaction of *H pylori*, we checked *vac*A (s1a, s1b, s1c, s2, m1, m1T, m2), *ice*A1, *ice*A2 and *cag* A.

RESULTS: Between October 2000 and April 2002, 88 patients with bleeding peptic ulcers (males/females: 60/28, gastric ulcers/duodenal ulcers: 55/33), 81 patients with non-bleeding peptic ulcers (males/females: 54/27, gastric ulcers/duodenal ulcers: 45/36) and 37 patients with chronic gastritis (males/females: 24/13) were enrolled in this study. In patients with bleeding peptic ulcers, non-bleeding peptic ulcers and chronic gastritis, 45 patients (51%), 71 patients (88%) and 20 patients (54%) respectively were found to have positive *H pylori* infection ($P<0.001$). In patients with bleeding peptic ulcers, non-bleeding peptic ulcers and chronic gastritis, polymerase chain reaction for *H pylori* infection was positive in 54 patients (61%), 70 patients (86%) and 20 patients (54%) respectively ($P<0.001$). The sensitivity, positive predictive value and diagnostic accuracy of mucosal polymerase reaction for *H pylori* infection were significantly lower in patients with bleeding peptic ulcers (84%, 79% and 81%) than in patients with non-bleeding peptic ulcers (99%, 99% and 98%) ($P<0.001$, $P<0.01$ and $P<0.001$ respectively). The sensitivity, negative predictive value and diagnostic accuracy of mucosal polymerase reaction for *H pylori* were significantly lower in patients with bleeding peptic ulcers (84%, 83% and 81%) than in patients with chronic gastritis (100%, 100% and 100%) ($P = 0.02$, $P = 0.02$ and $P = 0.001$).

CONCLUSION: Mucosal polymerase chain reaction for detecting *H pylori* infection is not reliable in patients with bleeding peptic ulcers.

Key words: *Helicobacter pylori* infection; Bleeding peptic ulcers; Mucosal polymerase chain reaction

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INTRODUCTION

In the past two decades, *Helicobacter pylori* (*H pylori*) has been confirmed to be linked to chronic gastritis, peptic ulcers, gastric cancer and MALT-lymphoma[1]. The diagnosis of *H pylori* infection can be divided into invasive and non-invasive methods[2]. Endoscopic biopsy is needed in the invasive method. On the other hand, non-invasive methods are more convenient and equally accurate in diagnosing *H pylori* infection in patients with non-bleeding peptic ulcers.

Eradication of *H pylori* in patients with bleeding ulcer may virtually prevent recurrence of both the disease and its complications[3-5]. Therefore, accurate diagnosis of *H pylori* infection is essential in the management of peptic ulcer bleeding. So far, there is no single test that is considered optimal for the diagnosis of *H pylori* infection[5].

In patients with peptic ulcer bleeding, the accuracy of invasive and non-invasive tests is often disappointing[6,7]. Non-invasive tests for *H pylori* infection are more accurate than invasive tests in patients with bleeding peptic ulcers[7]. However, there are some limitations in these non-invasive tests. So far, there are three popular non-invasive tests for *H pylori,* namely ELISA-based serology, ^{13}C- UBT (^{13}C- UBT) and stool antigen test[8-10]. High sensitivity of the ^{13}C- UBT (>92%), serological test (>95%), and stool antigen test (>90%) has been reported in non-bleeding peptic ulcers[7,10]. The ^{13}C- UBT is expensive and samples need to be checked from a special laboratory[5]. ELISA serology may remain positive several years after eradication of *H pylori*[11,12]. Therefore, it cannot reflect the present infection. The stool antigen test is not reliable in patients with bleeding peptic ulcers[13-15].

Polymerase chain reaction (PCR) is an established method for *H pylori* infection[16-19]. It is very sensitive and accurate in diagnosing *H pylori* infection as compared with other invasive techniques in patients with non-bleeding peptic ulcers[19-21].

So far, there has been only one study with a small sample size of patients concerning mucosal PCR for diagnosing *H pylori* infection in patients with bleeding peptic ulcers[20]. Therefore, clarifying the role of mucosal PCR in patients with bleeding peptic ulcers is needed. The objective of this study was to evaluate the sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy of the mucosal PCR test in patients with bleeding peptic ulcers as compared to patients with non-bleeding peptic ulcers and chronic gastritis.

MATERIALS AND METHODS

Patients were admitted to the trial if they had bleeding peptic ulcers (hematemesis or tarry stool within three days), non-bleeding peptic ulcers and chronic gastritis during endoscopic examination at our division. Only ulcers over 5 mm in size in patients with peptic ulcers were considered for inclusion in this study. Patients were excluded from the study with the following conditions: usage of antibiotics or proton pump inhibitor (PPI) within four weeks of enrollment, inability or unwillingness to give written informed consent; gastric malignancy, bleeding tendency (platelet count less than 50 000/mm^3, prothrombin time less than 30%, or taking anticoagulants), pregnancy or lactation.

Possible complications of endoscopic treatment and biopsy were discussed with the patients and their relatives and written informed consent was obtained before the trial. An experienced gastroenterologist Lin *et al* performed all hemostatic treatments. The study was approved by the Clinical Research Committee of the Veterans General Hospital Taipei.

During endoscopic examination, we used endoscopic injection or heater probe thermocoagulation if there was bleeding (oozing or spurting) or non-bleeding visible vessels at the ulcer base. Thereafter, we took four biopsy specimens from the greater curvature of gastric antrum: one for rapid urease test (RUT), one for histology, a third for *H pylori* culture and a fourth for PCR test, and four specimens from the greater curvature of gastric body: one for RUT, one for histology, a third for *H pylori* culture and a fourth for PCR test. RUT, *H pylori* culture, and histology were completed within 48 h after enrollment. Two specimens from each patient were stored at -70 ℃ until analyzed for PCR test.

H pylori isolates were cultured from gastric biopsy specimens as in our previous studies[22,23]. Positive culture was identified by positive reaction for catalase, urease and oxidase activities. The isolates were cultured at 37 ℃ on brain heart infusion (BHI) agar plates supplemented with 7% horse blood (containing nalidixic acid 10 μg/mL, trimethoprim 5 μg/mL, vancomycin 3 μg/mL, and amphotericin 2 ug/mL) with 120 mL/L CO_2 and a high humidity.

RUT was checked with an in-house urease test at room temperature for color change for up to 24 h after addition of the sample. The test was defined as positive if the color changed from yellow to red. For histological examination, sections of paraffin-embedded specimens were routinely stained with hematoxyline-eosin and Giemsa for detecting *Helicobacter*-like microorganisms by an experienced pathologist Li *et al* who was blinded to the study.

The method of performing PCR was described in our previous studies[22,23]. In brief, lysates of gastric mucosa biopsy specimens were used for PCR. DNA was extracted from gastric biopsy specimens according to the method described by Boom[24]. Biopsy specimens were then homogenized in guanidinium isothiocyanate, using a sterile micropestle. DNA was extracted, washed and eluted in 100 μL of 10 mmol/L Tris-HCL (pH 8.3). Two microliters of the eluted DNA were used for each PCR reaction.

The oligonucleotide primers for PCR amplification of specific segments are shown in Table 1[25,26]. For *vacA* evaluation, the PCR program comprised 35 cycles of denaturation (at 94 ℃ for 1 min), annealing (at 56 ℃ for 2 min), extension (at 72 ℃ for 1 min), and one final extension (at 72 ℃ for 10 min). For *cagA*, amplification was performed with 35 cycles of denaturation (at 94 ℃ for 1 min), annealing (at 56 ℃ for 2 min), extension (at 72 ℃ for 1 min), and one final extension (at 72 ℃ for 5 min). For *iceA* amplification, amplifications were performed with 40 cycles of denaturation (at 95 ℃ for 30 s), annealing (at 50 ℃ for 45 s), extension (at 72 ℃ for 45 s) and one final extension (at 72 ℃ for 10 min).

Table 1 Oligonucleotide primers used for *cagA* , *vacA* and *iceA* genotyping

Region detected	Primer designation	Primer sequence	PCR product (bp)
s1 and s2	VA1-F	5′ATGGAAATACAACAAACACACC3′	259/286
	VA1-R	5′CTGCTTGAATGCGCCAAACTTTATC3′	
s1a	SS1-F	5′GTCAGCATCACACCGCAAC3′	190
s1b	SS3-F	5′AGCGCCATACCGCAAGAG3′	187
s1c	S1C-F	5′CTYGCTTTAGTRGGGYTA-3′	213
m1	VA3-F	5′GGTCAAAATGCGGTCATGG3′	290
	VA3-R	5′CCATTGGTACCTGTAGAAAC3′	
m1T	m1T-F	5′GGCCACAATGCAGTCATGG3′	290
	m1T-R	5′CTCTTAGTGCCTAAAGAAACA3′	
m2	VA4-F	5′GGAGCCCCAGGAAACATTG3′	352
	VA4-R	5′CATAACTAGCGCCTTGCAC3′	
iceA1	iceA1F	5′GTGTTTTTAACCAAAGTATC3′	247
	iceA1R	5′CTATAGCCASTYTCTTTGCA3′	
iceA2	iceA2F	5′GTTGGGTATATCACAATTTAT3′	229
	iceA2R	5′TTRCCCTATTTTCTAGTAGGT3′	
lcagA	lcagAD008	5′ATAATGCTAAATTAGACAACTTGAGCGA3′	297
	lcagAR008	5′TTAGAATAATCAACAAACATCACGCCAT3′	

If any two of the primers in the mucosal PCR tests were positive, the PCR test was defined as positive. If only one test was positive, we would repeat the PCR test. If there was still only one positive primer, it was defined as equivocal. If all primers were negative, it was defined as negative.

The gold standard for positive *H pylori* infection, as suggested by the Maastricht Consensus Report 1997[27], was determined by a positive culture of *H pylori* or both a positive histological examination and a positive RUT.

The χ^2 test with or without Yates's correction, Fisher's exact test and ANOVA test were used when appropriate to compare the sensitivity, specificity, positive and negative predictive values and diagnostic accuracy among three groups. P value<0.05 was defined as statistically significant.

RESULTS

Between October 2000 and April 2002, a total of 102 patients with bleeding peptic ulcers, 87 patients with non-bleeding peptic ulcers and 37 patients with chronic gastritis were considered in this study. In patients with bleeding peptic ulcers, 14 patients were excluded due to: unwillingness to cooperate (n = 6), gastric malignancy (n = 3) and usage of PPI before enrollment (n = 5). A final total of 88 patients with bleeding peptic ulcers were enrolled (males/females: 60/28, mean age: 67.9 years, 95% CI: 65.2-70.7 years). In patients with non-bleeding peptic ulcers, six patients were excluded from the study due to: unwillingness to cooperate (n = 5) and usage of PPI (n = 1). A final total of 81 patients with non-bleeding peptic ulcers were enrolled (males/females: 54/27, mean age: 65.7 years, 95% CI: 64.2-67.4 years). A final total of 37 patients with chronic gastritis were enrolled (male/female: 24/13, mean age: 65.7 years, 95% CI: 61.7-69.7 years) (P>0.1 among three groups concerning age and sex ratio). The locations of ulcers in patients with bleeding peptic ulcers and non-bleeding peptic ulcers are as follows: gastric ulcer/duodenal

ulcer: 55/33 and 45/36 respectively (P>0.1). The stigmata of recent hemorrhage in patients with bleeding peptic ulcers are as follows: spurting hemorrhage in six cases, oozing hemorrhage in four cases, non-bleeding visible vessels in 35 cases, blood clots in 10 cases, pigmented spots in 15 cases and clean ulcer bases in 18 cases. We used heater probe thermocoagulation in 40 cases and endoscopic injection with epinephrine in 15 cases to stop the bleeding.

In patients with bleeding peptic ulcers, mucosal PCR achieved the highest positive rate when compared with the urease test, histology and culture (Table 2). However, the difference was not statistically significant.

Table 2 Positive urease test, histology, bacterial culture and mucosal PCR in patients with bleeding and non-bleeding peptic ulcers

	Bleeding peptic ulcers (n = 88, %)	Non-bleeding peptic ulcers (n = 81, %)	CG (n = 37, %)
Urease test	42/88 (48)	67/79 (85)	18/37 (49)
Histology	41/86 (48)	61/80 (76)	14/30 (47)
Culture	44/76 (58)	68/72 (94)	16/35 (46)
PCR[b]	54/88 (61)	70/81 (86)	20/37 (54)

[b]P<0.001 between bleeding peptic ulcers and non-bleeding peptic ulcers, and between non-bleeding peptic ulcers and chronic gastritis (CG).

In patients with bleeding peptic ulcers, non-bleeding peptic ulcers and chronic gastritis, the mucosal PCR tests were positive in 54 patients (61%), 70 patients (86 %) and 20 patients (54%) respectively (P<0.001 between bleeding peptic ulcers and non-bleeding peptic ulcers, and between non-bleeding peptic ulcers and chronic gastritis). The positive rates of the urease test, histology, and culture are described in Table 2. According to the predefined criteria, 45 patients (51%), 71 patients (88%) and 20 patients (54%) were *H pylori* positive in those with bleeding peptic ulcers, non-bleeding peptic ulcers and chronic gastritis respectively (P<0.001 between bleeding peptic ulcer *vs* non-bleeding peptic ulcer and between non-bleeding peptic ulcer and chronic gastritis).

The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of mucosal PCR were 84%(95%CI: 83.9-84.1%), 77%(95%CI: 76.9-77.1%), 79%(95% CI: 78.9-79.1%), 83% (82.9-83.1%) and 81% (80.9-81.1%) respectively in patients with bleeding peptic ulcers (Table 3), and 99%(95%CI: 98.9-99.1%), 90%(89.9-90.1%), 99%(98.9-99.1%), 90% (89.9-90.1%) and 98% (97.9-98.1%) respectively in patients with non-bleeding peptic ulcers, and 100%, 100%, 100%, 100% and 100% respectively in patients with chronic gastritis. The sensitivity, positive predictive value and diagnostic accuracy of mucosal polymerase reaction for *H pylori* were significantly lower in patients with bleeding peptic ulcers (84%, 79% and 81%) than in patients with non-bleeding peptic ulcers (99%, 99% and 98%) (P<0.001, P<0.01 and P<0.001 respectively). The sensitivity, negative predictive value and diagnostic accuracy of mucosal polymerase reaction for *H pylori* were significantly lower in patients with bleeding peptic ulcers (84%, 83% and 81%) than in patients with chronic gastritis (100%, 100% and 100%) (P = 0.02, P = 0.02 and P = 0.001).

The presence of blood in the stomach did not influence the sensitivity of mucosal PCR significantly. In patients with bleeding peptic ulcers, the sensitivity of mucosal PCR in patients with presence of blood in the stomach (21/25, 84%) was comparable to that in patients without bleeding in the stomach (17/20, 85%) (P>0.1).

DISCUSSION

The results of this study showed that mucosal PCR test was not effective in assessing *H pylori* infection in patients with peptic ulcer bleeding. Taking the invasive tests as the gold standard, the sensitivity of mucosal PCR was 15% lower in patients with peptic ulcer bleeding than that in patients with non-bleeding peptic ulcer.

The European *H pylori* Study Group and the US Food and Drug Administration proposed that the gold standard for the evaluation of *H pylori* infection should consist of at least two tests that differ from the ones being examined[4,28]. In this study, we used three independent tests (culture, RUT and histology) as the gold standard for the evaluation of *H pylori* infection. Our gold standard met the requirement of the European and FDA criteria.

In this study, we used mucosal PCR instead of cultured isolates. The positive culture rate was lower than that of mucosal PCR[16]. If we had used culture instead of PCR, the positive rate might have been underestimated. In addition, according to mucosal PCR studies of Weiss *et al* and Kobayashi *et al*, the sensitivity (94% and 100%) and specificity (100% and 100%) of PCR were better than other diagnostic tests[18,19]. Multiple infections of *H pylori* have been very common, and sampling error may occur while interpreting the culture results[16,17,23].

In patients with peptic ulcer bleeding, the prevalence of *H pylori* infection remains controversial. Gisbert *et al*[29] reported a 97.5% infection rate in their series. On the other hand, other authors have reported markedly reduced prevalence, ranging between 20% and 70%[6,30]. The conflicting results of these studies may be due to different detection methods. For example, rapid urease tests were used in most studies. It had a low sensitivity for detecting *H pylori* in patients with peptic ulcer bleeding[19,31]. Histology, culture and stool antigen tests have also been found to have a low diagnostic yield in patients with peptic ulcer bleeding[6,14,20,26]. In addition, some factors (e. g., antibiotics, PPI) might cause a false negative result.

Histology has been found to be better than urease test in patients with peptic ulcer bleeding[6]. Its positive rate was 61-89% in these patients[6]. In our study, the positive rate of histology was 48%.

Table 3 Results of PCR test in patients with chronic gastritis, non-bleeding and bleeding peptic ulcers (PU)

Patients	PCR results	*Hp* (+)	*Hp* (-)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Chronic gastritis	Positive	20	0	100	100	100	100	100
	Negative	0	17	(99.9-100.1)	(99.9-100.1)	(99.9-100.1)	(99.9-100.1)	(99.9-100.1)
Non-bleeding PU	Positive	70	1	99	90	99	90	98
	Negative	1	9	(98.9-99.1)	(89.9-90.1)	(98.9-99.1)	(89.9-90.1)	(97.9-98.1)
Bleeding PU	Positive	38	10	84[b]	77	79[d]	83[a]	81[f]
	Negative	7	33	(83.9-84.1)	(76.9-77.1)	(78.9-79.1)	(82.9-83.1)	(80.9-81.1)

[b]P<0.001 *vs* non-bleeding, P = 0.02 *vs* chronic gastritis; [d]P<0.01 *vs* non-bleeding; [f]P = 0.001 *vs* chronic gastritis, P<0.001 *vs* non-bleeding; [a]P=0.02 *vs* chronic gastritis. Hp+: either positive *H pylori* culture or both positive rapid urease test and histology. PPV: positive predictive value, NPV: negative predictive value.

In this study, the mucosal PCR test was lower in sensitivity, positive predictive value, and diagnostic accuracy in patients with bleeding peptic ulcers as compared to those with non-bleeding peptic ulcers. Our study was consistent with that of Gonzalo *et al*[20]. In their series, the positive rate of mucosal PCR in bleeding peptic ulcers was 20% lower than that of non-bleeding peptic ulcer. In our series, the positive rate of mucosal PCR was 25% lower than that of non-bleeding peptic ulcer. Similar findings exist with the urease test, histology and culture. In spite of these findings, mucosal PCR achieved the highest positive rate in patients with bleeding peptic ulcers in our series.

Blood in the stomach would reduce the accuracy of some tests, probably owing to constituents cross-reacting in the EIA or the effect of albumin[6,14]. In our study, patients with blood in the stomach did not influence the mucosal PCR result significantly. In patients with bleeding peptic ulcers, the sensitivity of mucosal PCR in patients with the presence of blood in the stomach (21/25, 84%) was similar to that of patients without blood in the stomach (17/20, 85%) (P>0.1). Our finding was compatible with that of Archimandritis *et al*.

In conclusion, the mucosal PCR test is not reliable in diagnosing *H pylori* infection in patients with bleeding peptic ulcers.

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