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

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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 vacC (s1α, s1β, s1c, s2, m1, m1T, m2), iceA1, iceA2 and cagA.

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 ulcer/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, we found positive *H. pylori* infection in 84% (95% confidence interval: 77.0-90.7) of patients, 83% (95% confidence interval: 75.0-90.7) of patients and 81% (95% confidence interval: 71.0-89.7) of patients with bleeding peptic ulcers, non-bleeding peptic ulcers and chronic gastritis, respectively. The sensitivity, negative predictive value and diagnostic accuracy of mucosal polymerase chain reaction for *H. pylori* were significantly lower in patients with bleeding peptic ulcers (84%, 79% 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

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[6].

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, 13C-UBT (13C- UBT) and stool antigen test[8-10]. High sensitivity of the 13C- UBT (>92%), serological test (>95%), and stool antigen test (>90%) has been reported in non-bleeding peptic ulcers[7,10]. The 13C- 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].

Polimerase 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; gastritis malignancy, bleeding tendency (platelet count less than 50 000/mm², 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 culture test and a positive culture of H. pylori, a third for histology, and a fourth for PCR test. The mucosal PCR tests were performed after the above procedures. These specimens from each patient were stored at -70 °C 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 °C 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 μg/mL) with 120 mL/L CO2 until analyzed for PCR test.

RESULTS

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 χ2 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.

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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, 8 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 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
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                 | 54/88 (61)                        | 70/81 (86)                            | 20/37 (54)    |

\(^bP<0.001\) 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 peptic bleeding 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% 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\(^{[42,33]}\). 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\(^{[41]}\). If 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\(^{[18,20]}\).

In patients with peptic ulcer bleeding, the prevalence of \(H\) pylori infection remains controversial. Gisbert et al\(^{[39]}\) 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%\(^{[30]}\). 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)    | (98.9-90.1) | (97.9-98.1) | (97.9-98.1)  |
| Bleeding PU       | Positive    | 38         | 10         | 83%            | 77             | 79      | 83      | 81           |
|                   | Negative    | 7          | 33         | (83.9-84.1)    | (76.9-77.1)    | (78.9-79.1) | (82.9-83.1) | (80.9-81.1)  |

\(^bP<0.001\) vs non-bleeding, \(P = 0.02\) vs chronic gastritis; \(^aP<0.01\) vs non-bleeding; \(^fP=0.01\) vs chronic gastritis, \(P<0.001\) vs non-bleeding; \(^dP=0.02\) 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.\(^6\). 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.\(^1\).

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

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