

DETECTION OF *H. PYLORI* IN DYSPEPTIC PATIENTS AND CORRELATION WITH CLINICAL OUTCOMES

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Abstract. The objectives of this study were to evaluate the methods used to diagnose *Helicobacter pylori* infection in gastric biopsies, and to evaluate the correlation between *H. pylori* infection and clinical outcomes. Gastric biopsies, obtained from 210 patients, were evaluated for *H. pylori* by culture, a commercial rapid urease test (RUT, Pronto Dry) and histological examination. A true positive result was either the culture or both the RUT and histological examination were positive. The results showed a *H. pylori* infection rate of 44.3% (93/210). The sensitivities, specificities, positive predictive values and negative predictive values were 88.2, 100, 100, and 91.4 % by the culture; 95.7, 98.3, 97.8, and 96.6% by RUT; and 96.8, 59.8, 59.8, and 65.7% by histological examination, respectively. The prevalences of *H. pylori* in non-ulcer dyspepsia (NUD), peptic ulcer dyspepsia (PUD) and gastric cancer (GCA) patients were 41.2, 57.9 and 70.6%, respectively. The χ^2 -test showed that GCA patients were significantly more frequent infected with *H. pylori* than NUD patients ($p < 0.05$). Our study indicates that the RUT method was highly sensitive, specific and appropriate for routine clinical use.

INTRODUCTION

Helicobacter pylori is a gram-negative curved-to-spiral microaerobic bacteria, considered an important etiological agent in the development of gastritis, peptic ulcers and gastric carcinoma (Ansorg *et al*, 1991; Parsonnet, 1994; Logan *et al*, 2001). At present, there are several techniques available for the detection of *H. pylori* (Fabre *et al*, 1994; Heatley, 1995; Kisa *et al*, 2002). On routine laboratory examination in Thailand, the rapid urease test (RUT) and histological examination are widely used for the diagnosis of this fastidious microorganism. The gold standard for diagnosing *H. pylori* infection is the culture technique, but the method is problematic because the microorganism is slow-growing, fastidious and requires a special growth media (Hazell *et al*, 1989; Heatly, 1995); consequently, the culture method is not used in the routine laboratory. The objective of this study was

to compare the RUT and histological examination methods to the culture method for the diagnosis of *H. pylori* infection in gastric biopsies.

Although there are several reports on the correlation between *H. pylori* infection and clinical outcomes, the results remain unclear and show discrepancies. We therefore determined to investigate *H. pylori* infection and its correlation with non-ulcer dyspepsia [(NUD); gastritis (GT)], peptic ulcer dyspepsia [(PUD); duodenal ulcers (DU) and gastric ulcers (GU)] and gastric cancer (GCA) in dyspeptic patients. The research should help physicians to understand the pathogenesis and planing of treatment.

MATERIALS AND METHODS

Patients and endoscopy

Two hundred and ten consecutive patients with dyspeptic symptoms who underwent upper gastrointestinal endoscopy were included in this study. They were recruited from the Endoscopy Unit of Srinagarind Hospital, Faculty of Medicine, Khon Kaen University between February 2002 and February 2004. The subjects were diagnosed as non-ulcer dyspepsia (NUD),

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peptic ulcer dyspepsia (PUD), gastric carcinoma (GCA) and other gastrointestinal diseases (GERD, duodenitis, etc). There were 99 males and 111 females with an age range of 18 to 88 years (mean 48.5 years).

We excluded patients who had antibiotic therapy, bismuth treatment, proton pump inhibitors, or H2-blockers within the previous month. Informed consent was obtained from each patient before being included in the study.

Biopsy specimens

Three gastric mucosal biopsy specimens from the antrum and the corpus were obtained from each patient and divided into three parts. Both antral and corpus specimens were used for culture, the rapid urease test (RUT) and histological examination.

Culture

The culture was performed according to Hazell *et al* (1989) with modification. Briefly, each antral and corpus specimen was immediately placed into transport media and brought to the laboratory within 2 hours, and stored under cold conditions. The biopsy specimens were homogenized in 200 µl of normal saline and cultured on 7% human blood agar (Difco, Detroit, Michigan, USA) containing the supplement SR147 (5 mg/l trimethoprim + 10 mg/l vancomycin + 5 mg/l amphotericin B + 5 mg/l cefsulodin (SR147, OXOID). The plates were incubated at 37°C under microaerophilic conditions (5% O₂, 10% CO₂, 85% N₂) and were examined after 4 and 7 days of incubation. Characteristic colonies of *H. pylori* were confirmed by Gram staining, oxidase, catalase, and urease tests.

Commercial rapid urease test (RUT, Pronto Dry test)

The RUT was performed according to the manufacturer's instructions (Medical Instruments Corporation, Solothurn, Switzerland). Briefly, one antral and one corpus specimen were directly inoculated onto the commercial RUT agar gel. The results were observed and recorded within 24 hours: a positive was indicated when the color changed from yellow to pink.

Histological examination

One antral and one corpus biopsy were

fixed in 10% buffered formalin, processed, then embedded in paraffin. Four sections of 3-4 µ thick were stained with modified Warthin-Starry stain for identification of *H. pylori* (Cohen and Laine, 1997; Li *et al*, 2004). The presence of spiral organisms on any of the slides was considered positive for *H. pylori*.

Analysis of test results

Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) for the test were analyzed in comparison with true positive criteria. The chi-square test and Fisher exact test were used for statistical analysis of *H. pylori* infection and clinical outcomes. $p < 0.05$ was considered statistically significant.

The criteria for a true positive of *H. pylori* result was considered as having a positive result on either the culture or RUT and the histological examination (Pajares-Garcia, 1998; Liao *et al*, 2003).

RESULTS

Comparison of culture, RUT, and histological examination for the diagnosis of *H. pylori*

H. pylori was detected by culture, RUT and histological examination in 82 (39%), 93 (44.3%) and 122 (58.1%) patients, respectively. Regarding true positive test criteria, *H. pylori* infection was found in 44.3% (Table 1). Sixty-seven, (31.9%) samples were positive by all three diagnostic methods, 26 (12.4%) by at least two meth-

Table 1
Positive detection rates of *H. pylori* infection in 210 dyspeptic patients detected by culture, RUT and histological examination.

Diagnostic method	No. of <i>H. pylori</i> infections (%)
Culture	82 (39)
RUT	93 (44.3)
Histological examination	122 (58.1)
True positive ^a	93 (44.3)

^a = culture positive or both urease and histological examination positive

Table 2
Numbers and percentages of *H. pylori* infections detected by the three diagnostic methods.

Culture	RUT	Histological	No. of infections (%)	Evaluation criteria ^a
+	+	+	67 (31.9)	TP
+	+	-	3 (1.4)	TP
+	-	+	4 (1.9)	TP
-	+	+	19 (9)	TP
-	+	-	4 (1.9)	FP
-	-	+	47 (22.4)	FP
-	-	-	66 (31.4)	TN
			210	93(44.3)%

TP = True positive, TN = True negative, FP = False positive

^a = TP, culture positive or both urease and histological examination positive

Table 3
Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of the three diagnostic methods.

Diagnostic method	% (no. of positive samples/no. tested)				Accuracy (%)
	Sensitivity	Specificity	PPV	NPV	
Culture	88.2 (82/93)	100 (117/117)	100 (82/82)	91.4 (117/128)	95
RUT	95.7 (89/93)	98.3 (113/115)	97.8 (89/91)	96.6 (113/117)	96
Histological examination	96.8 (90/93)	59.8 (70/117)	59.8 (90/137)	65.7 (70/73)	76

Table 4
Diagnosis and *H. pylori* infection corresponding to the true positive criteria^a.

Diagnosis	No. of patients (%)	<i>H. pylori</i> infection (%)
Non-ulcer dyspepsia	165 (78.6)	68/165 (41.2)
Peptic ulcer dyspepsia	19 (9)	11/19 (57.9)
Gastric cancer	17 (8.1)	12/17 (70.6)
Others	9 (4.3)	2/9 (22.2)
Total	210	93

^a= culture positive or both urease and histological examination positive

ods, and 51 (24.3%) by only one method (Table 2). The results of the sensitivity, specificity, PPV and NPV indicated that histological examination and RUT were more sensitive methods than culture. However, the histological examination had the least specificity, PPV and NPV compared to the RUT and the culture. The RUT was highly sensitive and specific, but had a lower specificity than culture (Table 3).

Correlation of *H. pylori* infection with gastrointestinal disease

Gastric biopsies were obtained from 210 patients: 165 (78.6%) patients had non-ulcer dyspepsia (NUD), 19 (9%) PUD, 17 (8.1%) gastric cancer (GCA) and 9 (4.3%) other gastrointestinal diseases. Of the 210 patients, 93(44.3%) patients had *H. pylori* infection. Sixty-eight (41.2%) of the 165 patients with NUD had *H.*

pylori infection, 11 (57.9%) of 19 patients with PUD had *H. pylori*, 12 (70.6%) of 17 patients with GCA had *H. pylori*, and 2 (22.2%) of 9 patients with other gastrointestinal diseases had *H. pylori* (Table 4). *H. pylori*-infection was more predominant among patients with gastric cancer than those with PUD and NUD. The χ^2 test showed that GCA patients were significantly more frequent infected with *H. pylori* than NUD patients ($p < 0.05$), whereas, PUD patients were more often infected with *H. pylori* than NUD patients, but this did not reach statistical significance.

DISCUSSION

Various standard methods for the detection of *H. pylori* infection in human gastric mucosa were evaluated (Fabre *et al*, 1994; Cutler *et al*, 1995; Lage *et al*, 1995). Like other research, different methods yielded different rates of detection of *H. pylori* infection (Kokkola *et al*, 2000; Logan *et al*, 2001). Our study showed that *H. pylori* detection rates by culture, RUT, and histological examination were 39, 44.3, and 58.1%, respectively (Table 1). Among the three diagnostic methods, RUT was highly sensitive, specific, and had a high PPV and NPV compared to a true positive (Table 3).

Our results from the RUT method agreed well with previous studies (Onders, 1997; Castro-Fernandez *et al*, 2004). The RUT method was not only high in those parameters but gave a much faster result when compared to histological examination and culture. This is an advantage to the physician to diagnose *H. pylori* before the patient is discharged from the endoscopy room (Lim *et al*, 2004).

Our histological examination was highly sensitive but not sufficiently specific (Table 3). This finding disagreed with those of others (Onders, 1997; Logan *et al*, 2001; Kisa *et al*, 2002). In general, the histological examination has a sensitivity and specificity of 90-95% (Onders, 1997). Other researchers used Giemsa stain or hematoxylin and eosin for histological examination (Onders, 1997; Kisa *et al*, 2002) but we used a modified Warthin-Starry stain, which is good at visualizing the organism, but sometimes may give a granular appearance of silver

impregnation, which looks like the organism. These can lead to false positive biopsy readings (Madan *et al*, 1988). Therefore, histological staining should be improved to increase the specificity of *H. pylori* detection in the future.

Culture is the gold standard for the detection of *H. pylori* infection (Hazell *et al*, 1989; Heatley, 1995; Malfertheiner *et al*, 2002). Although, it is a difficult method, time consuming and plagued with false negatives, it is a prerequisite for further studies of this organism, such as classification, antibiotic resistance and other comparative studies (Chowdhury *et al*, 1991). A previous study reported a more significant correlation between the RUT and culture (0.90) than between histological findings and culture (0.80) (Kawanishei *et al*, 1995); therefore, it is possible to improve the sensitivity of the culture method, by preventing contamination.

Most studies have described the prevalence of *H. pylori* infection, since its incidence is difficult to determine (Talley *et al*, 1993). The prevalence of *H. pylori* infection has varied in previous studies from different countries (Talley *et al*, 1993; Goh, 1997; Apostolopoulos *et al*, 2002), depending on the environment, host and laboratory detection methods (*ie* by geographical area, detection methods, patient selection, socioeconomic status, age range and period of study) (Perez-Perez *et al*, 1990; Gilboa *et al*, 1995; Heathley, 1995; Malfertheiner *et al*, 2002).

In Thailand, the detection of *H. pylori* infection also varies between hospitals. The detection of *H. pylori* infection at Srinagarind Hospital was 44.3%, whereas it varied between 46 and 62% at other hospitals around the country (Arnantapunpong, 1999; Phiphitaporn, 1999; Pankongngam, 2001). Our study showed a lower *H. pylori* infection compared to other hospital reports, because we used the criteria for positive in *H. pylori* infection as culture positive or both RUT and histological examination positive, instead of using only one method.

H. pylori has been identified as the cause of chronic gastritis, peptic ulcer disease, gastric cancer and MALT (Ansorg *et al*, 1991; Parsonnet, 1994; Logan *et al*, 2001) but the pathogenesis of *H. pylori* infection is not completely understood. Several researchers have shown that sev-

eral putative virulence factors may contribute to mucosal damage (Blaser and Berg, 2001; Montecucco and Rappouli, 2001). Recent studies have shown that the major reservoir of *H. pylori* is man and that the principal mode of transmission is person to person. The route of transmission however remains obscure. There have been some reports of a correlation between the high prevalence of *H. pylori* infection and the high rate of GCA, especially in populations with endemic *H. pylori* infection in childhood (Correa *et al*, 1990). Logan *et al* (2001) reported that about 15% of *H. pylori* infected individuals will develop peptic ulcers or gastric cancer as the long term consequence of infection.

Our study showed that *H. pylori* infection in NUD, PUD and GCA was 41.2, 57.9 and 70.6%, respectively, and *H. pylori* infection was significantly associated with GCA patients. Other hospitals in Thailand have reported that *H. pylori* infection was either significantly or non-significantly associated with PUD, but have not studied the prevalence of *H. pylori* infection in GCA patients (Arnantapunpong, 1999, Phiphitaporn, 1999; Pankongngam, 2001).

In conclusion, the RUT is a very simple, highly sensitive and specific method, thus it is appropriate for routine clinical use. A correlation between GCA patients and *H. pylori* infection was significantly different compared to GT patients.

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