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Density of *Helicobacter pylori* may affect the efficacy of eradication therapy and ulcer healing in patients with active duodenal ulcers

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Abstract

AIM: To evaluate the association of pre-treatment *Helicobacter pylori* (*H. pylori*) density with bacterial eradication and ulcer healing rates in patients with active duodenal ulcer.

METHODS: One hundred and four consecutive duodenal ulcer outpatients with *H. pylori* infection ascertained by gastric histopathology and ^{13}C-urea breath test (UBT) were enrolled in this study. *H. pylori* density was graded histologically according to the Sydney system (normal, mild, moderate, and marked). In each patient, lansoprazole (30 mg b.i.d.), clarithromycin (500 mg b.i.d.) and amoxicillin (1 g b.i.d.) were used for 1 week, then 30 mg lansoprazole once daily was continued for an additional 3 weeks. Follow-up endoscopy was performed at 4 weeks after completion of the therapy, and UBT was done at 4 and 8 weeks after completion of the therapy.

RESULTS: The *H. pylori* eradication rates were 88.9 %/100.0 %, 94.3 %/100.0 %, and 69.7 %/85.2 %; and the ulcer healing rates were 88.9 %/100.0 %, 94.3 %/100.0 %, and 63.6 %/77.8 % (intention-to-treat/per protocol analysis) in the mild, moderate, and marked *H. pylori* density groups, respectively. The association of pretreatment *H. pylori* density with the eradication rate and ulcer healing rate was both statistically significant (P=0.013/0.006 and 0.002/<0.001, respectively; using results of intention-to-treat/per protocol analysis).

CONCLUSION: Intragastric bacterial load may affect both the outcome of eradication treatment and ulcer healing in patients with active duodenal ulcer disease.

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INTRODUCTION

Helicobacter pylori (H. pylori) has been considered as the main cause of chronic gastritis and peptic ulcer[1]. It has been identified in 90-95 % of patients with duodenal ulcer and 60-80 % of patients with gastric ulcer[2]. Strong evidence has also shown an association between the eradication of *H. pylori* infection and the cure of peptic ulcer diseases[3-5].

Recommendations and guidelines for *H. pylori* eradication therapy have been developed rapidly during recent years. The global trend of eradication therapy has now shifted to proton pump inhibitor (PPI)-based triple therapy (a PPI and two different anti-microbials)[6], which can assure rapid symptom relief, improve ulcer healing, and reduce ulcer recurrence[7,8]. However, some cases failed to heal with this advanced PPI-based triple therapy. Patient compliance and antibiotic resistance are currently regarded as the major causes of eradication failure[9]. Several researchers have also revealed that high antral density of *H. pylori* may increase the rate of ulcer recurrence and be related with low eradication rate after triple therapy[10-12]. Intragastric bacterial load might be another factor affecting the success of eradication therapy[12]. Therefore, we conducted this study to investigate the association of *H. pylori* density in the stomach with the efficacy of eradication therapy and ulcer healing in patients with active duodenal ulcers.

MATERIALS AND METHODS

Patients

From January 2000 to June 2002, consecutive outpatients with endoscopically verified active duodenal ulcers (5 mm in diameter or larger) at Cathay General Hospital were enrolled in this study. All panendoscopic examinations were performed and interpreted by the same group of experienced endoscopists. We only enrolled patients with positive diagnosis of *H. pylori* infection proven by both histological examinations and ^{13}C-urea breath tests (UBT). To avoid interference in evaluating the status of *H. pylori*, the following patients were initially excluded before endoscopy: those who had ingested bismuth, antibiotics, anti-secretory medication, or PPI during the 4 weeks prior to the beginning of the study; those who used non-steroidal anti-inflammatory drugs; those who were pregnant or immuno-compromised, and those who had a history of gastric surgery or a previous attempt to eradicate *H. pylori*. The patients with coexisting gastric ulcers or gastric cancer were also excluded. All procedures were performed after obtaining informed consent from the patients.

The patients enrolled were treated with the same regimen of 30 mg lansoprazole b.i.d. plus 500 mg clarithromycin b.i.d. and 1 g amoxicillin b.i.d. for 7 days. After 1 week of anti-*H. pylori* therapy, 30 mg lansoprazole once daily was continued for an additional 3 weeks. From then on, either PPI or anti-secretory H_2-blocker was avoided; only oral antacids were taken for symptomatic relief when necessary. The second endoscopic examination was performed 4 weeks after completion of the therapy.

Histology

During the first endoscopic examination, three biopsies were taken from the gastric antrum (one near the incisura and the

other two on the greater and lesser curvature, 2 cm within the pyloric ring)[13]. On the follow-up endoscopic examination, two additional specimens were taken from the greater curvature of the gastric body due to the possibility of patchy distribution of *H. pylori* after eradication therapy. An "Olympus GIF-XQ 200' endoscope was used, the biopsy forceps were the "FB 25-K" type. The specimens were stained with hematoxylin-eosin and with a modified Giemsa stain. Then they were examined by experienced histopathologist who was unaware of the clinical diagnosis for the existence of *H. pylori* of the patients. The version of the visual analogue scale in the updated Sydney system was used to grade the density of *H. pylori* (4 grades: normal, no bacteria; mild, focal few bacteria; moderate, more bacteria in several areas; and marked, abundance of bacteria in most glands)[14]. If the density varied, the highest grade of density in the specimens was selected.

Urea breath test

UBTs were performed before the start of therapy and on two separate occasions, at 4 and 8 weeks after the cessation of therapy, respectively. The ^{13}C-urea used was 100 mg 99 % ^{13}C-labelled urea produced by the Institute of Nuclear Energy Research, Taiwan. Patients drank 100 ml of fresh milk as the test meal. The procedure was modified from the European standard protocol for the detection of *H. pylori*[15,16]. We chose 3 per mil for the cut-off level of the rise in the delta value of $^{13}CO_2$ at 15 min after the ingestion of ^{13}C-urea. UBT was defined as positive when the excess $\delta^{13}CO_2$ was above this value. By using this cut-off, the sensitivity and specificity of UBT were 97 % and 95 %, respectively[16].

Eradication of *H. pylori* infection was defined as negative results on UBT and histology tests at 4 weeks after the cessation of therapy or negative results on two UBTs at both 4 and 8 weeks after the cessation of therapy[17]. Healing of ulcers was defined as complete disappearance of the ulcer and non-healing as persistence of ulcers, even if the size had decreased[18].

To be included in the intention-to-treat analysis, the infection of *H. pylori* had to be confirmed using both UBT and histological examination results. When protocol violations which were likely to influence the response variable or its assessment occurred, patients were excluded from the per protocol analysis.

Statistical analysis

Chi-square or Fisher' s exact test was used to evaluate the qualitative data. ANOVA test was applied for the quantitative data. The eradication rates of *H. pylori* and healing rates of duodenal ulcers in the three groups of different *H. pylori* densities were compared using chi-square test or Fisher' s exact test and assessed on the basis of both intention-to-treat and per protocol analysis. The 95 % confidence intervals (95 % CI) were calculated. The relationship between *H. pylori* eradication and duodenal ulcer healing was evaluated by Fisher' s exact test. *P* value less than 0.05 was considered significant.

RESULTS

Patients

One hundred and ten patients were initially included in the study, but 6 patients were later excluded due to their negative results of *H. pylori*. We enrolled 104 patients who fulfilled the inclusion criteria. Table 1 shows the demographic and other baseline characteristics of the patients divided by the grade of *H. pylori* density. None of differences in variables was statistically significant. Among them, 12 patients did not complete the study because of poor compliance of taking medications (3 cases) or refusal to receive the second endoscopy (9 cases). Because we wanted to observe the results of both the eradication therapy and the ulcer healing, the patients who refused the second endoscopy were excluded. Therefore, 92 patients were included for per protocol analysis.

Table 1 Clinical characteristics of patients

	H. pylori density		
	Mild (*n*=36)	Moderate (*n*=35)	Marked (*n*=33)
Sex (male / female)	23 / 13	25 / 10	23 / 10
Age (years, Mean ±S.D.)	45.6±10.3	44.1±12.7	47.2±13.4
Smoking(yes / no)	18 / 18	20 / 15	18 / 15
Ulcer size			
< 1 cm	32	32	30
1-2 cm	4	2	3
>2 cm	0	1	0
Ulcer number			
One	34	32	30
Two	2	3	3
Drop outs	4	2	6

Eradication rate and the density of H. pylori

The overall eradication rates were 84.6 % (95 % CI: 75.9-90.7 %) in the intention-to-treat analysis set and 95.7 % (95 % CI: 88.6-98.6 %) in the per protocol set. The eradication rates of the three grades of bacterial densities had a downward trend as the bacterial load became denser (Table 2). The association was statistically significant (*P*=0.013 and 0.006, by intention-to-treat and per protocol analysis).

Table 2 Eradication rates and the density of *H. pylori*

	Eradication rates	95% CI	*P* value
Intention-to-treat			
H. pylori density			
Mild	32 / 36 (88.9%)	73.0 – 96.4 %	
Moderate	33 / 35 (94.3%)	79.5 – 99.0 %	
Marked	23 / 33 (69.7%)	51.1 – 83.8 %	
Total	88 / 104 (84.6%)	75.9 – 90.7 %	0.013[a]
Per protocol			
H. pylori density			
Mild	32 / 32 (100.0%)	86.7 – 100.0 %	
Moderate	33 / 33 (100.0%)	87.2 – 100.0 %	
Marked	23 / 27 (85.2%)	65.4 – 95.2 %	
Total	88 / 92 (95.7%)	88.6 – 98.6 %	0.006[b]

Notes: [a]Based on Chi-square test; [b]Based on Fisher' s exact test.

Healing rates of duodenal ulcer and the density of H. pylori

The healing rates of active duodenal ulcer are shown in Table 3. According to both of the intention-to-treat and per protocol analysis, the differences in the ulcer healing rates of the three grades of *H. pylori* densities were statistically significant (*P*=0.002 and <0.001, respectively). As the bacterial density increased in the stomach, the healing rate of duodenal ulcer decreased.

Relationship between H. pylori eradication and duodenal ulcer healing

Table 4 shows the positive impact of *H. pylori* eradication on duodenal ulcer healing. The association was statistically significant (*P*<0.001). Active ulcers of 97.7 % of the patients with successful *H. pylori* eradication had healed at the time of

the second endoscopic examination.

Table 3 Healing rates of duodenal ulcer and the density of *H. pylori*

	Healing rates	95% CI	*P* value
Intention-to-treat			
H. pylori density			
Mild	32 / 36 (88.9%)	73.0 – 96.4 %	
Moderate	33 / 35 (94.3%)	79.5 – 99.0 %	
Marked	21 / 33 (63.6%)	45.1 – 79.0 %	
Total	86 / 104 (82.7%)	73.8 – 89.2 %	0.002[a]
Per Protocol			
H. pylori density			
Mild	32 / 32 (100.0%)	86.7 – 100.0 %	
Moderate	33 / 33 (100.0%)	87.2 – 100.0 %	
Marked	21 / 27 (77.8%)	57.3 – 90.6 %	
Total	86 / 92 (93.5%)	85.8 – 97.3 %	<0.001[b]

Notes: [a]Based on Chi-square test; [b]Based on Fisher's exact test.

Table 4 Relationship between *H. pylori* eradication and duodenal ulcer healing

	H. pylori	
	Eradicated (*n*=88)	Not eradicated (*n*=4)
Duodenal ulcer		
Healed	86 (97.7%)[a]	0 (0.0%)
Not healed	2 (2.3%)	4 (100.0%)

[a]*P*<0.001 *vs* not eradicated group, based on Fisher's exact test.

DISCUSSION

H. pylori is an important factor in the pathogenesis of duodenal ulcer. Researchers have shown the eradication of *H. pylori* not only prevents ulcer recurrence but also aids ulcer healing[19]. Eradication of *H. pylori* has changed the natural history of duodenal ulcer and has become a standard therapy for duodenal ulcer patients[3, 4, 10].

At present, short-term (7-day) triple therapies including a PPI and two antibiotics (clarithromycin, and amoxycillin or a nitroimidazole compound) are considered as the first line in anti-*H. pylori* regimens[12]. However, despite of the high efficacy of different anti-*H. pylori* treatment schemes, a proportion of patients, varying from 10 % to 25 %, fails the first attempt to eradicate *H. pylori*[9]. Patient compliance and antibiotic resistance are currently regarded as the key factors affecting the outcome of treatment[9,12]. The evidence for other possible factors associated with lower eradication rates is somewhat sparse and equivocal. Nevertheless, several reports revealed that the patients with higher intragastric *H. pylori* load had reduced eradication rates. This association was found in both bismuth and PPI-based triple therapies[9-12]. Therefore, pre-treatment bacterial density was used by some authors to predict the success of *H. pylori* eradication in patients with chronic duodenal ulcer[10,11].

Our working hypothesis was that "the higher the intragastric *H. pylori* colonization, the less effective the short-term triple therapy". In our study, an inverse relationship between successful eradication and intragastric bacterial load was found, which is compatible with the results of other studies[9-12]. Increased bacterial density of *H. pylori* was associated with a significant reduction in the eradication rate after anti-*H. pylori* treatment. This correlation provides additional support for the importance of bacterial density as a factor in *H. pylori* eradication.

With more *H. pylori* inhabiting the antrum, the gastric mucosa will suffer greater damage by this bacteria[20, 21]. The denser infection of *H. pylori* associated with greater antral inflammation may cause lower somatostatin expression, leading to higher levels of gastrin and acid production, which may therefore predispose the duodenum to ulceration[22]. Alam *et al*[21] have shown that the prevalence of duodenal ulcer increased with increasing *H. pylori* density, and a greater likelihood of ulceration was noted among patients with high concentrations of *H. pylori*. Effective *H. pylori* eradication was found to induce good responses in peptic ulcer healing[18]. Treiber *et al* [23] reported successful *H. pyl*ori eradication therapy accelerated peptic ulcer healing even without concomitant acid suppression. In several trials, *H. pylori* eradication not only did result in greater duodenal ulcer healing, but also dramatically decreased ulcer recurrence rate 12 months following treatment[24]. The cure rates of *H. pylori* infection could be expected more than 90 % using standard triple therapy according to the Maastricht consensus guidelines, and the effective *H. pylori* eradication therapy could achieve rapid peptic ulcer healing in more than 90 % of subjects[23,25].

In our study, 86/88 cases (97.7 %) in the *H. pylori* eradication group had complete healing of ulcers, which were proven by the results of follow-up endoscopy. The association between *H. pylori* eradication and ulcer healing was statistically significant. Therefore, a positive impact of *H. pylori* eradication on peptic ulcer healing is concluded.

Intragastric bacterial load can affect the success of *H. pylori* eradication therapy, and *H. pylori* eradication can influence the healing of duodenal ulcer. Thus, the healing of duodenal ulcer might be associated with the *H. pylori* density in the stomach. According to our results, *H. pylori* density was revealed to be inversely related to ulcer healing. We asked every patient enrolled to receive follow-up endoscopic examinations so that we could observe the healing process of duodenal ulcer directly. Although some patients refused the second endoscopic examination, our results were still able to confirm the assumed association mentioned above.

Identification of pre-treatment predictors of eradication therapy is important in clinical practice. Therapeutic regimens may be tailored to individual patients according to their particular conditions. Despite the fact that high *H. pylori* density might adversely influence the efficacy of eradication therapy, patients with high intragastric bacterial loads were found to benefit from an extension of eradication regimen from 1 to 2 weeks[9]. Prolonging duration of the standard triple therapy was proposed as a way to overcome the unfavorable effects caused by high *H. pylori* density and proved to be effective[9,12].

Most patients (101/104 cases, 97.1 %) included in this study had good drug compliance. Bacterial culture and drug sensitivity test to assess the effect of antibiotic resistance were not performed. Therefore, we could not demonstrate the influence of drug resistance on eradication therapy by our results. Although some authors mentioned the resistance to antimicrobials could be considered as a statistical event and estimated as a certain proportion of the *H. pylori* population in the stomach[26], the relationship between antibiotic resistance and bacterial load of *H. pylori* has not been studied. Further investigation is warranted to elucidate this issue. The culture and susceptibility testing of *H. pylori* are time-consuming and expensive, and they are rarely performed in most developing countries[27]. In Taiwan, they are not routinely performed either. Our study may thus provide a practical option to identify the pre-treatment predictors of eradication therapy and ulcer healing when information on drug sensitivity is not available.

In conclusion, our findings reveal that pre-treatment high bacterial density of *H. pylori* in the stomach adversely affects

the success of eradication therapy and influences the healing of ulcers in patients with active duodenal ulcers, and effective *H. pylori* eradication is also shown to be significantly associated with duodenal ulcer healing. Identifying patients with high bacterial loads before treatment and making adjustments of therapeutic regimens accordingly may further improve the efficacy of eradication therapy.

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