EVALUATION OF THE SEVERITY OF *HELICOBACTER PYLORI* INFECTION WITH UREASE TEST: ITS CORRELATION WITH HISTOPATHOLOGY AND BACTERIAL DENSITY

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In 69 patients, the severity of *Helicobactor pylori* (*H. pylori*) infection was evaluated by bacterial density of tissue implants and inflammatory responses by histology. The specimens were taken from gastric angle and antrum (greater and lesser curvature sides) by gastroduodenal endoscopy. In urease test, the severity was measured in 3 grades according to color change of the agar: those change are within 30 minutes (grade 3), 30 minutes to 3 hours (grade 2), and 3 to 6 hours (grade 1), respectively; while the grade 0 indicated no color change occurring 6 hours after tissue inoculation. The severity of infection was assessed according to the bacterial density under high power microscopic fields (Gram's stain). Grade 0 indicated no bacterium seen; grade 1, only 1 to 10 bacteria at all fields; grade 2, 1 to 3 bacteria in each high power field; and grade 3 was 4 bacteria or more on average in each high power field. The degree of inflammatory response was evaluated by inflammatory cell infiltration (H & E stain) and classified into grade 0, 1 and 2, which indicated the inflammatory cell infiltration below 50%, between 50% and 75%, and above 75%, respectively. There are no positive relationships among urease test reaction time, bacterial density grading and degrees of inflammatory cell infiltration. Clinically, the reaction time of urease test cannot reflect the severity of *H. pylori* infection semi-quantitatively, either in terms of bacterial density or cellular inflammatory response.

Key words: *Helicobacter pylori*, enzymology, inflammation

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Since 1910, there has been virtually no debate on acid being the main factor in the pathophysiology of peptic ulcer. The discovery of *Helicobacter pylori* (*H. pylori*) not only appears to have created an alternative hypothesis for ulcer development, but it also sheds light on the common mysterious chronic inflammatory state of the gastric mucosa, antral gastritis type B. Stolte *et al.* have demonstrated a highly significant association between the degree of colonization and the severity and activity of gastritis in both the antrum and corpus from 1265 patients with *H. pylori*-associated gastritis[1]. Other authors, using more sophisticated morphometric techniques, have also found a significant correlation between the density of bacterial colonization and the degree of inflammation in antral specimens[2,3]. There were several methods in detecting *H. pylori*: urease test, bacterial culture, Gram's stain, histopathologic examination, urea breath test, polymerase chain reaction[4] and serologic test. However, some of these tests are time-consuming and costly. In a previous study, we compared the sensitivity and specificity among the urease test, Gram's stain, culture, and histology in the detection of *H. pylori* and the results disclosed less sensitivity (71%) and more time taken (4 to 5 days) in the culture method, while the urease test had the advantages

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of rapidity, high sensitivity (95%) and specificity (96%)[5]. Therefore, we conducted a further study trying to evaluate whether rapid urease test can successfully predict the bacterial density of *H. pylori* and inflammatory response induced.

MATERIALS AND METHODS

Patients and sterilization of endoscope

This study included 69 patients (39 men and 30 women, mean age: 39.7±15.3 years old) in whom gastroduodenoscopy was conducted as part of the investigation of dyspeptic symptoms. Of these 69 patients, there were 37 patients with peptic ulcer disease in the scar stage, and 32 with non-ulcer dyspepsia. Patients with coagulopathy, malignancy, or other major systemic disease were excluded from the study. The endoscope was cleaned with glutaraldehyde, and the biopsy channel and forceps were sterilized with an antiseptol solution between examinations. During endoscopic examination three sets of biopsies were taken from the lesser and greater sides of antrum (within 5 cm of the pylorus) and angle, respectively. Each set contained 3 biopsies. These were submitted in separate containers, one set for urease test, another set for Gram's stain, and the last one set for histopathologic examination.

Urease test

Once obtained, the specimen was immediately inserted into the Christensen urea agar (Difco). The urea agar contained 2% urea and phenol red; pH was set at 6.8. We observed the color change within 30 minutes, at 3 hours, and at 6 hours. The chemical reaction of the urease test was classified into 3 grade; the color change within 30 minutes (grade 3), 30 minutes to 3 hours (grade 2), 3 hours and 6 hours (grade 1). If no color change occurred six hours after inoculation, it was considered grade 0.

Bacterial density

The second set of specimens was stamped on a slide for Gram's stain with safranin O as the counter stain for direct observation of the curved rod shaped bacteria. The bacterial density was graded as follows: 0, when no bacterium was seen; grade 1: only 1 to 10 bacteria at all the visual fields; grade 2, on average of 1 to 3 bacteria at each high power field; and grade 3, over 4 bacteria on average at each high power field.

Histopathology examination

The third set of specimens was fixed with 5% formalin. These were embedded in paraffin for section and H & E stain. The examination of the histological specimens were by the same pathologist and were done consecutively. The pathologist was unaware of the other results. The degree of inflammatory cell infiltration was evaluated as grade 0 when inflammatory cell infiltration was below 50% of all visual fields; grade 1, when between 50% and 75%; and grade 2, when above 75% on average.

RESULTS

Table 1 showed the relationship between the grade of Gram's stain and the severity of inflammatory cell infiltration. If the tissue samples with negative staining of *H. pylori* were excluded, no correlation was found between the numbers of bacteria seem by Gram's stain and the severity of inflammatory cell infiltration. Table 2 disclosed that among 207 tissue samples examined by histopathology for inflammatory cell infiltration, 26 of 32 (81.2%) specimens that measured as grade 2 of severity had a positive urease test, and, there were 66.6% (66/99), and 18.4% (12/65) positive urease test in grade 1 and grade 0, respectively. There was a positive relationship between the urease test reaction times and the grades of inflammatory cell infiltration. However, after excluding the tissue samples with negative urease test results, no statistically significant correlation could be found between them. Table 3 showed that there was a positive correlation between the urease test reaction time and the grading of bacterial density by Gram's stain. However, if the tissue samples with negative urease test results were excluded, the correlation was not significant either.

DISCUSSION

The urease test was first described by McNulty and Wise[6] and then applied by others[5,7-9]. It is an indirect method to detect *H. pylori* and is convenient in clinical practice. The results can occur from within minutes to a

Table 1. The Relationship between the Density of *Helicobacter pylori* Colonisation and the Severity of Inflammation

Pathology	Bacterial density			
	Gr III	Gr II	Gr I	Gr 0
Gr II	7*	7	11	7
Gr I	26	20	20	33
Gr 0	6	2	4	53

*numbers of tissue samples

Table 2. The Relationship between the Rapidity of Urease Test and the Severity of Inflammation

Pathology	Rapidity of urease rest			
	Gr III	Gr II	Gr I	Gr 0
Gr II	20*	4	2	6
Gr I	57	4	5	33
Gr 0	7	3	2	53

*numbers of tissue samples

Table 3. The Relationship between the Rapidity of Urease Test and Bacterial Density

Bacterial density	Rapidity of urease test			
	Gr III	Gr II	Gr I	Gr 0
Gr III	31*	6	2	1
Gr II	25	2	4	1
Gr I	29	2	4	2
Gr 0	2	1	1	93

*numbers of tissue samples

few hours and interpretation is easy[6]. For the purpose of excluding the interference of other organisms which may have weak urease activity, we ended the interpretation of color change after 6 hours of tissue insertion[5].

Both urease test and Gram's stain were reported to have a high sensitivity, high specificity and to consume less time[5]. Moreover, the routine use of urease test enables gastroenterologists to diagnose *H. pylori* infection easily even without microbiological expertise. There is consensus on the role of *H. pylori* colonization in causing antral inflammation in patients with duodenal ulcer, gastric ulcer, and non-ulcer dyspepsia[1-3,10]. Hazell *et al.* found that the urease test could be used as a marker of bacterial colonization and gastritis[8]. However, Marshall *et al.* disclosed no significant association between bacterial density seen under microscopy and the rate at which the Campylobacter-like Organism (CLO) test would be positive[7]. In our study, Christensen urea agar was used as the mediam for rapid urease test. Lee *et al.* reported Temmler CUT test was more sensitive and gave the fastest reaction than others[11]. Whether the different media applicated and/or different criteria in interpretation the time of color changes in urease test influence the results remained to be determined. The topographic distribution of *H. pylori* infection may also have an effect upon the outcome of the study by using the tissue samples obtained from endoscopy biopsy[12].

We conclude that rapid urease test is a very fast, useful method to detect *H. pylori* infection, and that it can predict the presence of inflammatory response; but it cannot reflect bacterial density and inflammatory response semiquantitatively.

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以尿素酶評估幽門螺旋桿菌感染嚴重度：其與細菌密度及發炎反應之相關性

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幽門螺旋桿菌（簡稱 *H. pylori*）之感染已被認爲是胃炎的主要原因之一，而尿素酶法是檢測 H. pylori 感染快速而準確的方法。本研究之主要目的是評估尿素酶法是否能作爲反應 H. pylori 感染嚴重程度的指標。共有69位接受胃鏡檢查之病人（男39人，女30人，平均年齡39.7±15.3歲），其中32位爲非潰瘍性消化不良，37位爲瘢痕期潰瘍病人。在胃鏡檢查中，以切片鉗夾取胃竇大小彎及胃角處組織作尿素酶測定，玻片組織壓印之革蘭氏染色及病理 H.E. 染色。將尿素酶法之反應時間定爲0級（6小時內仍未變色），一級（3至6小時內變色），二級（30分至3小時內變色），三級（30分鐘內變色）。病理組織之發炎反應分爲零級（發炎細胞平均占視野50%以下），一級（50－75%），二級（75%以上）。革蘭氏染色下細菌密度分爲三級（平均高倍每個視野下至少4株以上），二級（平均1－3株），一級（所有視野下1－10株），0級（看不到 H. pylori）。結果發現尿素酶法之反應時間與組織發炎反應及細菌密度皆無相關。結論：內視鏡切片之尿素酶測定法可以判定 H. pylori 感染之有無及發炎反應之存在與否，但無法作爲評估 H. pylori 感染嚴重程度之指標。

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