# *H. pylori* Infection and the Correlation to Chronic Active Gastritis Detected by the Histological Criteria of the Sydney System

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Abstract : In Thailand, chronic active gastritis and chronic atrophic gastritis have not been given frequent mention. Our aim is to present the relationships of the many variables in histological classification of chronic bacterial gastritis by the Sydney System. This paper presents the prevalence of chronic active gastritis by studying the degree of *H. pylori* infection detected by the Modified Toluidine Blue staining (MTBs) and histological grading, using the Sydney System. A total of 355 dyspeptic patients who underwent endoscopy in Kawila hospital, Chiang Mai, northern Thailand from January 1996 to January 1998, were included in the study. Overall, 284 patients (80.3 %) had an *H. pylori* infection. Diagnosis varied from normal, chronic gastritis, and acute gastritis to chronic active gastritis in 11 (3.1 %), 95 (26.8 %), 0 (0 %),

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249 (70.1 %) patients respectively. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of neutrophils presented with lymphocytes are 85.2, 90.1, 97.2, and 60.4 %, respectively. We conclude from the study that chronic active gastritis is a very common condition in Thailand. If chronic active gastritis is present, *H. pylori* is usually detected, and the degree of infection is well correlated to the degree of inflammation.

**เรื่องย่อ** : *H. pylori* **และความสัมพันธ์กับ** Chronic Active Gastritis **วินิจฉัยโดยใช้** Histological Criteria of the Sydney System

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ภาวะ chronic active gastritis และ chronic atrophic gastritis มีกล่าวถึงกันน้อยในประเทศไทย ทั้งที่เป็นภาวะที่พบได้บ่อย การวินิจฉัยสามารถทำได้โดยอาศัย classification หลายแบบ แต่ที่กำลังมีการศึกษากันอย่างกว้างขวางในขณะนี้ได้แก่ Sydney System รายงานฉบับนี้ได้นำเสนอถึง ความชุกในการติดเชื้อ *Helicobacter pylori* โดยการย้อมด้วย Modified Toluidine Blue staining (MTBs) ร่วมกับการศึกษาความสัมพันธ์ของการติดเชื้อกับความรุนแรงของการอักเสบในเยื่อบุกระเพาะอาหาร การศึกษานี้ทำขึ้นที่โรงพยาบาลกาวิละ จังหวัดเชียงใหม่ ตั้งแต่ มกราคม 2539 ถึง มกราคม 2541 จำนวนผู้ป่วยที่ได้รับการตรวจวินิจฉัยโดยการส่องกล้องระบบทางเดินอาหารส่วนต้น 355 คน พบว่ามีการติดเชื้อ *H. pylori* 284 คน (ร้อยละ 80.3) ผลการตรวจวินิจฉัยทางพยาธิวิทยามีตั้งแต่ normal 11 คน (ร้อยละ 3.1), chronic gastritis 95 คน (ร้อยละ 26.8), acute gastritis 0 คน (ร้อยละ 0), chronic active gastritis 249 คน (ร้อยละ 70.1) ตามลำดับ ถ้าเราพบ neutrophils ร่วมกับ lymphocytes จะพบว่ามีโอกาสติดเชื้อ *H. pylori* สูงโดยจะมี sensitivity ร้อยละ 85.2, specificity ร้อยละ 90.1, positive predictive value (PPV) ร้อยละ 97.2 และ negative predictive value (NPV) ร้อยละ 60.4 สรุปได้ว่า chronic active gastritis เป็นภาวะที่พบได้บ่อยในผู้ป่วยชาวไทย และเมื่อเราพบภาวะนี้ มักจะมีการติดเชื้อ *H. pylori* ร่วมด้วยเสมอ นอกจากนี้ความรุนแรงของการอักเสบยังแปรผันตามปริมาณของเชื้อที่ตรวจพบอย่างชัดเจน

## INTRODUCTION

*Helicobacter pylori* infection is associated with many gastroduodenal diseases such as peptic ulcer and gastric cancer. From the theory of gastric carcinogenesis, end stage chronic atrophic gastritis, so-called gastric atrophy, is believed to be a precancerous lesion. *H. pylori* may play the major role in this point since it has been proved to be the major cause of chronic active gastritis resulting in chronic atrophic gastritis and gastric atrophy.[1,2] From this theory, WHO declared in 1994 that *H. pylori* was a carcinogen.[3] Anyone who is infected with this organism faces an eight to nine times greater risk of developing gastric cancer compared with non-infected people.[4] Detection of this organism is still an attractive topic for discussion.

*H. pylori* detection is simply classified into non-invasive and invasive technique. For the non-invasive technique such as serum IgG antibody against *H. pylori* and Urea Breath Test (UBT) are safe and represent the whole-stomach study. But, according to the impractical reasons of non-invasive technique in Thailand such as low specificity IgG or unavailable UBT, invasive test might be more appro-priate. For the invasive test, gastroscopic-based with gastric biopsy is currently accepted for detection of *H. pylori* infection. Several diagnostic methods (for example; urease test, special staining, and immune staining, etc.) have been reported for many years. We used histological criteria of the Sydney System classification[5,6] for grading the degree of chronic and active inflammation using H&E stain, as well as the degree of infection using the new staining method, Modified Toluidine Blue (MTBs), as the new cost-effective and simple method for detection of the infection.

## MATERIALS AND METHODS

From January 1996 to January 1998, 355 dyspeptic patients (median age 58 years, range 21 - 85 years, M/F = 129/226 (36.3 % /63.7 %)) were examined in Kawila hospital, Chiang Mai, northern Thailand. The following details were recorded: age, sex, past medical history, past history of dyspepsia or ulcer pain and medication, smoking and drinking habits, and family history. Gastroscopic examinations were performed on all patients. At least three fixed points were biopsied at the antrum, corpus and incisura angularis in order to represent the whole-stomach study. Tissue specimens were fixed immediately in buffered formalin solution prior to the staining process. Paraffin-processed sections were cut at three levels and stained with hematoxylin and eosin (H&E). A further section was taken from each set of biopsy specimens and stained with the Modified Toluidine Blue staining (MTBs).

**Modified Toluidine Blue staining (MTBs):** The reagent is prepared by controlling the pH of a Toluidine solution at strictly 5.4. The sections to be examined are cleaned in xylene four times, and fixed in alcohol four times. They are then rinsed in tap water, tapped in distilled water twice, and then placed in a Toluidine Blue solution for 10 minutes. Finally, they are rinsed in tap water, dehydrated in alcohol and cleaned by xylene, then cleared and mounted.

The background should be bright blue. The organisms are also blue and appear to be typically 0.5 mm wide, 1-3 mm long, curved spiral-rod in shape and confined in a mucus layer or just above

the epithelial layer.

In the study, all sections were independently coded, examined and scored for the presence of *H. pylori* by one of the authors (Nobutaka Yamada) without knowledge of the clinical information.

Sensitivity, specificity, PPV, NPV of MTBs: From the preliminary study of 319 patients, the sensitivity, specificity, PPV, NPV of MTBs were 99.2%, 100%, 100% and 100% respectively by immune staining method as standard.[7] The further collective data with resemblant result are in process of manuscript preparation.

The Sydney System: We used the histological criteria of the Sydney System classification for grading the degree of chronic and active inflammation, and the degree of infection.[5] The criteria were established in 1990 in order to standardize clinical information, including endoscopic and pathological findings of gastritis and the response of gastric mucosa to injury, so the information could easily be understood by generalists and specialists in all parts of the world. The method of collecting the gastric mucosa, which was revised in 1994, recommended the greater and lesser curvature biopsy instead of the anterior and posterior sites suggested in the original so-called Houston modifications of the Sydney System.[6] From the histological division of this system, the grading of acute and chronic gastritis can be done by morphological variables such as: inflammation grading by density of mononuclear cell infiltration, activity grading by neutrophil density, atrophy, presence or absence of intestinal metaplasia, and density of *H. pylori*-like organisms. The three-grade system of mild (1+), moderate (2+), and marked (3+) that was used represented an increment in severity of approximately one-third. Mononuclear cell (predominantly lymphocyte) infiltration meant chronic gastritis. Chronic inflammation alone, without neutrophil infiltration, was classified into chronic inactive gastritis (CIG).

This study focused on the degree of neutrophils infiltration presented in a background of chronic inflammation (lymphocyte, plasma cell, macrophage), so-called chronic active gastritis (CAG). Histological diagnosis of CAG was judged according to the highest score of inflammation and/or activity among the three biopsies.

Statistical analysis: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are calculated to indicate the pre-sence or absence of acute or chronic inflammation compared to the positivity of *H. pylori*. Correlation of the degree of infection and inflammation was measured by Kappa and coefficient of agreement.

## RESULTS

*H. pylori* infection and histological diagnosis: a total of 355 patients who underwent endoscopy were included in the study. The histological diagnosis and *H. pylori* status are shown in table 1. Overall, 284 patients (80.3 %) had an *H. pylori* infection. Diagnosis varied from normal, chronic gastritis, and acute gastritis to chronic active gastritis in 11 (3.1 %), 95 (26.8 %), 0 (0 %), 249 (70.1 %) patients respectively. Among the chronic active gastritis cases, 32 (12.8 %) patients had a mild infection, 50 (20.1 %) were moderate, and 167 (67.1 %) had marked infection.

Prevalence of gastric atrophy: the preva-

Table 1 Histological diagnosis and *H. pylori* status detected in 355 patients from Kawila Hospital, Chiang Mai, Thailand, graded by the histological criteria of the Sydney System

| Histology | *H. pylori* 0 (none) | 1 (mild) | 2 (moderate) | 3 (marked) | Total |
|---|---|---|---|---|---|
| WNL | 6 | 5 | 0 | 0 | 11 |
| CIG | 58 | 27 | 8 | 2 | 95 |
| AG | 0 | 0 | 0 | 0 | 0 |
| CAG (mild) | 1 | 22 | 6 | 3 | 32 |
| CAG (moderate) | 1 | 9 | 29 | 11 | 50 |
| CAG (marked) | 5 | 2 | 15 | 145 | 167 |
| Total | 71 | 65 | 58 | 161 | 355 |

WNL "within normal limits, CIG" chronic inactive gastritis, AG "acute gastritis, CAG" chronic active gastrits.

Table 2 Prevalence of gastric atrophy graded by the histological criteria of the Sydney System

| Atrophy | Frequency (n) | Percentage (%) |
|---|---|---|
| 0 (none) | 99 | 27.9 |
| 0.5* | 227 | 63.9 |
| 1 (mild) | 14 | 3.9 |
| 2 (moderate) | 12 | 3.4 |
| 3 (marked) | 3 | 0.8 |
| Total | 355 | 100 |

* = inconclusive,
1 + 2 + 3 = atrophy = 8.1 %.

Table 3 Accuracy of lymphocytes (chronic gastritis) as the predictor for *H. pylori* infection

| Lymphocyte | *H. pylori* Positive | Negative | Total |
|---|---|---|---|
| Positive | 279 | 65 | 344 |
| Negative | 5 | 6 | 11 |
| Total | 284 | 71 | 355 |

Sensitivity = 98.2 %, Specificity = 8.4 %, PPV = 81.1 %, NPV = 54.5 %.

Table 4 Prevalence of chronic active gastritis graded by the histological criteria of the Sydney System

| Chronic active gastritis | Frequency (n) | Percentage (%) |
|---|---|---|
| 0 (WNL or CIG) | 106 | 29.9 |
| 1 (mild) | 32 | 9 |
| 2 (moderate) | 50 | 14.1 |
| 3 (marked) | 167 | 47 |
| Total | 355 | 100 |

1+ 2 + 3 = chronic active gastritis = 70.1 %
WNL "within normal limits, CIG" chronic inactive gastritis.

Table 5 Accuracy of neutrophils together with lymphocytes (chronic active gastritis) as the predictor for *H. pylori* infection

| | *H. pylori* | | |
|---|---|---|---|
| Neutrophil | Positive | Negative | Total |
| Positive | 242 | 7 | 249 |
| Negative | 42 | 64 | 106 |
| Total | 284 | 71 | 355 |

Sensitivity = 85.2%, Specificity = 90.1 %, PPV = 97.2 %, NPV = 60.4 %

Table 6 Correlation between the degree of *H. pylori* infection and inflammation (chronic active gastritis) graded by the histological criteria of the Sydney System

| | | | *H. pylori* | | |
|---|---|---|---|---|---|
| Neutrophils | 0 (none) | 1 (mild) | 2 (moderate) | 3 (marked) | Total |
| 0 (none) | 58 | 30 | 9 | 0 | 97 |
| 1 (mild) | 7 | 18 | 9 | 3 | 37 |
| 2 (moderate) | 1 | 14 | 21 | 26 | 62 |
| 3 (marked) | 5 | 3 | 19 | 132 | 159 |
| Total | 71 | 65 | 58 | 161 | 355 |

Kappa = 0.5, and the coefficient of agreement = 64%

lence of atrophic change could only be detected in 8.1 per cent as shown in table 2.

The accuracy of lymphocytes as the predictor for *H. pylori* infection: sensitivity, specifi-city, positive predictive value (PPV) and negative predictive value (NPV) were 98.2, 8.4, 81.1, 54.5 %, respectively. The results are detailed in table 3.

The prevalence of chronic active gastritis: chronic active gastritis was found in 249 patients (70.1 %) patients as shown in table 4.

The accuracy of neutrophils combined with lymphocytes as the predictor for H. pylori infection: sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 85.2, 90.1, 97.2, and 60.4 %, respectively. The results are detailed in table 5.

The correlation between the degree of infection and inflammation: as shown in table 6, a good correlation between those two factors is found. Kappa is 0.5 while the coefficient of agreement is 64%.

## DISCUSSION

The Sydney System is a working formulation for the reporting and classification of gastritis by linking etiology, topography and morphology together. Its flexibility permits a standard scale for grading key morphological features that enable investigators to compare results, together with other classifications that are easily translated into the System's terminology.[5] In general, we used H&E staining for grading the degree of gastritis. This type of staining can be used for identifying *H. pylori*, but it appears to depend on the pathologist's individual experience. Thus, as we were able to detect the organism in about 50 - 90 per cent of cases with accuracy, special stains are recommended as an aid to identification. Many satisfactory methods are therefore available. The choice of stain, for example, modified Giemsa, Warthin-Starry or the new Genta stain, is a matter of local preference.[6] In the present study, we used MTBs for the detection and grading of *H. pylori* infection. With this simple and cost-effective technique, we found the prevalence of *H. pylori* infection to be 80.3 per cent of the patients of median age 58 years (table 1).

Atrophy in the oxyntic mucosa is closely linked to loss of acid secretion and to the development of intestinal metaplasia, which in turn is linked to an increased risk of gastric cancer. Extensive atrophy in antral mucosa, usually associated with intestinal metaplasia, also carries an increased risk of malignancy.[6] Our data show that chronic atrophic gastritis resulting in gastric atrophy is not especially common in Thailand, where the prevalence is 8.1 per cent (table 2). This finding might correlate to the relatively low prevalence of gastric cancer in Thailand country compared with other endemic areas of gastric cancer.

Chronic gastritis diagnosed by lymphocyte and plasma cells infiltration of the mucosa, suggesting the activation of antigen-specific cellular and humoral immunity, is a very common condition in Thai people. But we could not use lymphocytes as a good predictor for *H. pylori* infection due to low specificity (table 3). Unfortunately, we did not find acute gastritis on its own in this study. This finding might be from the rare occurrence of this transient condition.[5] Interestingly, we did find that chronic active gastritis is also common, with a prevalence of

70.1 per cent (table 4). This polymorph response, which represents a primary host defense mechanism against an invading pathogen, will be stimulated directly by products of *H. pylori*, including the water-soluble *H. pylori* neutrophil activating protein (HP-NAP), and indirectly following induction of the potent neutrophil chemotactic and activating peptide interleukin-8 and other cytokines involved in the inflammatory cascade.[8] When neutrophils were presented together with lymphocytes, so-called chronic active gastritis, *H. pylori* was detected in 97.2 % (sensitivity, specificity, PPV, NPV were 85.2, 90.1, 97.2 and 60.4 %, respectively [table 5]). Compared with the previous study that showed sensitivity, specificity, PPV, NPV as 86.7, 93.7, 96.2, and 79.5 %, respectively,[9] neutrophils were also used as an accurate indicator in the detection of *H. pylori*. This finding demonstrated the likely role of neutrophil-derived reactive oxygen species and protease and neutrophil "activity" is likely to be linked to tissue damage.[6] Furthermore, the present study showed a good positive correlation between the degree of infection and the degree of the inflammation scoring by the Sydney System (table 6). This finding has been mentioned previously[10] and may be useful in follow-up cases since the complete disappearance of both active and chronic gastritis is found after successful eradication.[11] In case that neutrophilic polymorphs are seen in a post-treatment biopsy but organisms are not apparent, a careful search for *H. pylori* using one of the special stains or immune stains should be carried out.[6]

As shown in table 2, the score "0.5" means " inconclusive", whether negative or weakly positive. This means that unclear interpretation in some instances is common. For this reason, the Sydney System which is used all around the world, is still problematic. Many intra-observer and inter-observer variations in the histopathological assessment are still under discussion.[12] International agreement on the interpretation and didactic session together with step-by-step instructions on scoring techniques are needed in order to unify the grading of chronic gastritis.

## CONCLUSION

By our technique, we conclude that chronic active gastritis is a very common condition in Thailand. If chronic active gastritis is present, *H. pylori* is usually detected. The degree of infection is well correlated to the degree of inflammation, and the non-infected stomach is usually classified as chronic inactive gastritis. These findings confirm the pathogenesis of chronic gastritis caused by *H. pylori* and might prone useful in clinical evaluation before and after *H. pylori* eradication.

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