

## Gastric cancer: Relation between histologic subtypes, location and *Helicobacter pylori* infection

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**Objective** : *To study the relation between histologic types, location of gastric cancer and H. pylori.*

**Design** : *Descriptive study*

**Setting** : *Department of Pathology, Faculty of Medicine, Chulalongkorn University.*

**Subjects** : *Patients diagnosed as gastric adenocarcinoma from January to December 1997.*

**Methods** : *Sex, age, and macroscopic features were evaluated included location, forms and growth pattern. Microscopic features, identified tumor cell types and the presence of Helicobacter pylori were studied and reviewed.*

**Results** : *There were 45 patients. The ages ranged from 32 to 86 years (mean age was 61.7 years). The male to female ratio was 1.25:1. There was no significant age difference between the men and women studied.*

*In approximately 29 (64.4%) cases, the tumor arose in distal areas (antrum/pylorus); in 13 (28.9%) cases it was detected in the body and in 3 (6.7%) cases it was located at proximal areas (cardia/fundus).*

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*In 31 (68.9%) cases, the tumor was an ulcerated type. Infiltrative macroscopic features were found in 11 (24.4%) cases, compared to 3 (6.7%) cases of a fungating gross appearance.*

*Twenty-five cases (55.6 %) of gastric cancer were classified as an intestinal type and 20 (44.4%) as a diffuse type.*

*H. pylori was identified in 14 (31.1%) cases. There was no significant association between infection and histological type of tumor, nor was there any significant association between infection and site of tumor or age.*

**Conclusion** : *There was no correlation between histologic types, location of gastric cancer and **Helicobacter pylori** in this study.*

**Key words** : *Gastric cancer, Lauren's classification, Tumor location, **Helicobacter pylori**.*

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นฤมล วิเศษโสภาส, พิสิฐ ตั้งกิจวานิชย์, พงษ์พีระ สุวรรณกุล, โรสริน จันทโรจวงศ์, สุธิดา อิงคตานุวัฒน์. ความสัมพันธ์ระหว่างลักษณะทางพยาธิวิทยาและตำแหน่งที่เกิดมะเร็งกระเพาะอาหารกับการติดเชื้อ *Helicobacter pylori*. จุฬาลงกรณ์เวชสาร 2540 ธันวาคม ; 41 (12): 889-99

วัตถุประสงค์ : เพื่อศึกษาความสัมพันธ์ระหว่างลักษณะทางพยาธิวิทยา และตำแหน่งที่เกิดมะเร็งกระเพาะอาหาร *Helicobacter pylori*

รูปแบบการวิจัย : การวิจัยเชิงพรรณนา

สถาบันการศึกษา : ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

การคัดเลือกตัวอย่าง : ชิ้นเนื้อที่ได้รับการวินิจฉัยมะเร็งกระเพาะอาหารชนิด adenocarcinoma ตั้งแต่เดือน มกราคม จนถึงเดือน ธันวาคม พ.ศ. 2540

ผลการศึกษา : ผู้ป่วยทั้งหมดที่ศึกษาจำนวน 45 ราย อายุระหว่าง 32 ปี ถึง 86 ปี (อายุเฉลี่ย 61.7 ปี) เป็นเพศชาย 25 ราย เพศหญิง 20 ราย คิดเป็นอัตราส่วนระหว่างชาย: หญิง = 1.25: 1 พบว่ามะเร็งเกิดที่ตำแหน่ง distal (antrum/pylorus) ร้อยละ 64.4 พบที่บริเวณ body ร้อยละ 28.9 ส่วนที่ตำแหน่ง proximal (cardia/ fundus) พบร้อยละ 6.7

ลักษณะทางกายวิภาคพบแบบ ulcerative จำนวน 31 ราย คิดเป็นร้อยละ 68.9 แบบ infiltrative พบ 11 ราย คิดเป็นร้อยละ 24.5 ส่วนที่เหลือเป็นแบบ fungating 3 รายคิดเป็นร้อยละ 6.7

ลักษณะทางจุลพยาธิวิทยาพบแบบ intestinal จำนวน 25 ราย (ร้อยละ 55.6) และอีก 20 ราย ที่เหลือพบเป็นแบบ diffuse คิดเป็นร้อยละ 44.5 โดยพบว่ามี การติดเชื้อ *Helicobacter pylori* ร่วม กับชนิด intestinal และแบบ diffuse อย่างละ 7 ราย

สรุป : ในการศึกษาพบว่ลักษณะทางพยาธิวิทยาและตำแหน่งที่พบมะเร็งกระเพาะอาหารไม่มีความสัมพันธ์กับการติดเชื้อ *Helicobacter pylori* การศึกษาความสัมพันธ์ระหว่างลักษณะทางพยาธิวิทยา และตำแหน่งที่พบมะเร็งกระเพาะอาหารกับการติดเชื้อ *Helicobacter pylori* จากการศึกษาจุลพยาธิวิทยาเพียงอย่างเดียวอาจไม่เพียงพอ ควรนำวิธีอื่นๆ ในการวินิจฉัยเชื้อ *H. pylori* มาเปรียบเทียบร่วมด้วย

Analysis of gastric carcinoma, one of the commonest cancers world wide<sup>(1)</sup>, has led to speculation about environmental factors that may be important in its pathogenesis<sup>(2)</sup>. The coexistence of chronic atrophic gastritis and carcinoma is common, but the etiopathogenic link between the two and the relative risk for malignancy in the former remain controversial<sup>(3-6)</sup>.

Since the discovery of *Helicobacter pylori* in 1983 much research has focused on this bacteria. This organism has become widely accepted as an important pathogen in the upper gastrointestinal tract. It is now considered the cause of type B chronic active antral gastritis, to be a critical factor in duodenal ulcer disease and, more recently, gastric ulcer, and also to be an essential co-factor in the development of the intestinal form of gastric carcinoma<sup>(7-8)</sup>. Several lines of evidence support an association between infection with *Helicobacter pylori* and gastric cancer. It is well established that *Helicobacter pylori* causes chronic gastritis, thereby, triggering the pathological changes that can be lead to gastric cancer<sup>(9)</sup>. Moreover, areas with a high prevalence of *Helicobacter pylori* infection also have a high prevalence of gastric carcinoma<sup>(10)</sup>. In contrast to the situation seen in peptic ulcer disease, it is not possible to confirm the relationship between gastric cancer and *Helicobacter pylori* by demonstrating disease cure after get rid off the organisms; gastric cancer is generally diagnosed too late for eradication therapy to be of any benefit. Evidence for the role of *Helicobacter pylori* in the development of gastric cancer primarily comes, therefore, from epidemiological studies.

Whether there is a difference between the intestinal and the diffuse types of gastric cancer in their association with *Helicobacter pylori* infection is less clear<sup>(8, 14-16)</sup>. Several of studies carried out

in gastric cancer patients did not subdivide their result on the basis of *Helicobacter pylori* infection and cancer location within stomach.

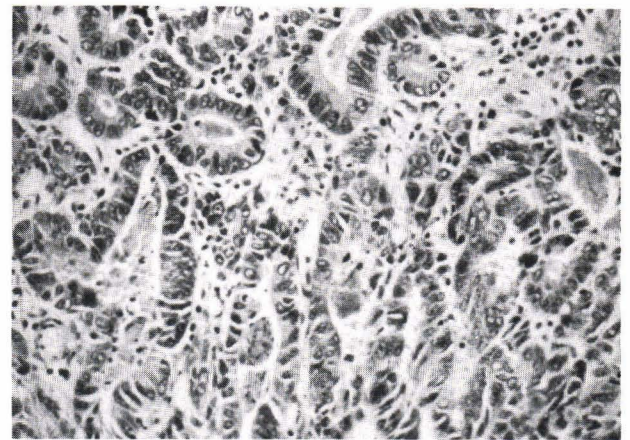
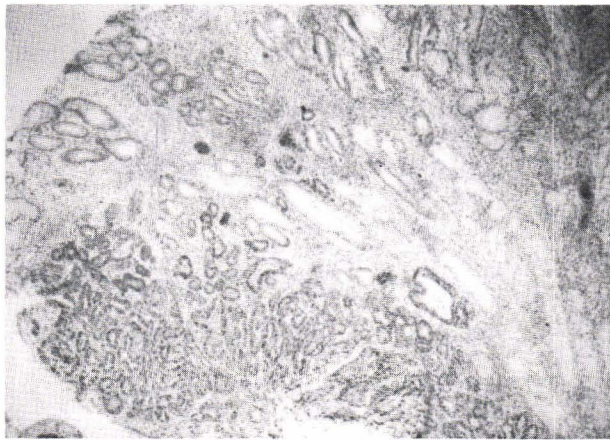
The purpose of this study was to examine the relations between *Helicobacter pylori* infection, location and histologic types of gastric cancer.

### Materials and methods:

Forty-five formalin-fixed gastrectomy specimens with histologically diagnosed as gastric adenocarcinoma at Department of Pathology, Faculty of Medicine, Chulalongkorn hospital between January to December 1997 were identified. Biopsy material was not included because it contained insufficient non-tumor tissue. In all cases, multiple specimens were taken from tumor, peritumor mucosa, and resected margins. Tumor site was recorded as follows: proximal (cardia/fundus, n= 3); body (n= 13); distal (antrum/pylorus, n= 29). Sex and age of patients were always analised. The specimens were fixed in 10% buffered formalin. The tumors were divided macroscopically into 1) a fungating or polypoid type in which the bulk of the mass protuded into the lumen of the stomach and was usually sessile and broad-based; 2) an ulcerative type in which the major part of its surface was eroded, often with a typically thickened raised margin and a base covered with slough; and 3) an infiltrative type with a thickened plaque-like growth or localized infiltration. Linitis plastica in which submucosal and muscular infiltration involved part or all of the stomach, was included in this type. Superficial carcinoma in which areas of malignant change were confined to the mucous membrane was excluded in this study. Pafaffin embedded histological sections were cut at 4 um thickness and stained with haematoxylin and eosin. Special stains including

Periodic acid- Schiff reaction with and without diastase digestion, mucicarmin and immunohistochemical techniques were performed whenever necessary. Some of these specimens were available for electron microscopy to confirm diagnosis of carcinoma.

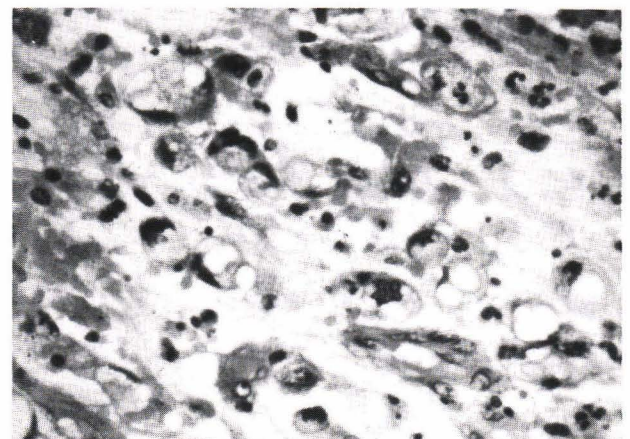
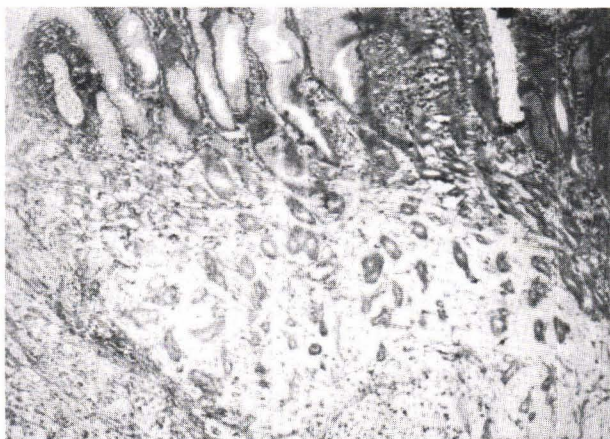
All sections were microscopically typed according to Lauren's classification into:



**Figure 1.** A, Low power photomicrograph of a gastric carcinoma, intestinal type. The lesion arises from a mucosa that shows the main component malignant gland-like structures. B, Close-up view of the intestinal type depicted in A.

b) Diffuse type, composed originally of dispersed cells that do not form recognizable glandular structures, tends to grow with an infiltrative pattern, and

may show variable degree of signet-ring cell formation, partly corresponding to the WHO's signet-ring cell type. (Figure 2).



**Figure 2.** A, Low-power photomicrograph of a diffuse type adenocarcinoma, there is diffusely infiltrate of tumor cells. B, Close-up view of the neoplasm composing of scattered individual cells that have clear, sometimes foamy cytoplasm and hyperchromatic nuclei.

Another classification of gastric carcinoma, Ming's classification<sup>(11)</sup>, based on pattern of growth and invasiveness, fall into two typed was recorded as follows: 1) expanding, where cells grow en masse and by expansion; and 2) infiltrative, where cells invade the stomach wall individually or in very small clusters.

All samples were examined independently by the authors for histological features. In instances when there was interobserver variation, disagreements resolved by review and discussion of the cases.



**Figure 3.** Modified Giemsa stain identified spiral bacteria typical of *Helicobacter pylori* within lumen of gastric gland. (X 600).

The occurrence of *Helicobacter pylori* was also assessed. In routine H&E stain *H. pylori* are not always properly identified because of flecks of mucous false-positive test results occur. Special stains, such as modified Giemsa stain, were therefore introduced (Figure 3). No attempt was made to differentiate the various subtypes of intestinal metaplasia.

Statistical analysis of the results was performed using the chi-square test with the Yates correction and Fisher's exact test.

### Results:

In total, 45 cases of gastric adenocarcinoma were included in the study. There were 25 men (55.6%) and 20 women (44.4%), giving a male to female ratio of 1.25: 1. The mean age of the patients was 61.7 years with a range of 32 to 86 years. There was no significant age difference between the men and women studied.

In 31 cases, the tumor was of an ulcerated type with 15 (48.4%) cases of an expanding pattern and 16 (51.6%) cases of an infiltrative pattern. An infiltrative macroscopic features was found in 11 cases with 3 (27.3%) of an expanding and 8 (72.7%) infiltrative pattern compared to 3 cases of a fungating gross appearance. All of which revealed an expanding pattern (100%).

Twenty-five cases (55.6 %) of gastric cancer were classified as an intestinal type and 20 (44.4%) cases as a diffuse type. There was no significant differences in the ages distribution of patients with regard to the different tumor types, but intestinal carcinoma was proportionately more common in men (Table 1).

*H. pylori* was identified in 14 ( 31.1%) cases. Among *H. pylori*-positive cases, no significant difference was found in intestinal cancer or diffuse cancer (28 vs. 28%). Prevalence of infection for the main tumor type is disclosed in Table 1.

**Table 1.** Characteristics of patients with the diffuse and the intestinal types of carcinomas and association of the tumor with *Helicobacter pylori* infection.

Characteristic	Intestinal cancer*	Diffuse cancer**
Mean age, years	63.7	57.1
<i>H. pylori</i> -positive	7 (28%)	7 (28%)

\* n = 25

\*\* n = 20

cancer type  $\chi^2 = 0.0324$ ; df= 1; not significant

**Table 2.** Location of Gastric cancer and its association with *Helicobacter pylori* infection.

Location	n	<i>H. pylori</i> -positive
Distal (antrum/pylorus)	29	8 (27.58%)
Body	13	6 (46.15%)
Proximal (cardia/fundus)	3	0 (0%)

\*\* Fisher's exact = 0.243 (p> 0.05; not significant)

When possible, tumors were also categorized on the basis of their location within the stomach. The tumor site distribution is shown in Table 2.

### Discussion:

Carcinoma of the stomach shows marked variations in incidence in differences countries, race, and sexes. Below age 35 to 40, the male: female ratio is about 1:1<sup>(17-19)</sup> or less<sup>(20)</sup> and increases up to a peak of 2.5 at age 60, then decreasing again<sup>(21)</sup>. If other parameters are evaluated in combination with age, then it appears that the diffuse type of carcinoma is more common

than the intestinal type in young people, particularly female<sup>(17-19, 22)</sup>.

This study indicates that most patients are over 50 years of age. There are only 9 cases defined as younger. Most of whom were diagnosed as a diffuse type, which is more frequent in women.

In terms of location, any area of the stomach can be effected: along the lesser curvature, predominantly in the antrum (52%), less frequently in the body (28%), and in 20% involved both areas<sup>(11)</sup>. Our cases show relevant data to the previous reports.

Because of complexity of the morphological features, many microscopic classifications have

been devised for gastric carcinoma. The World Health Organization (WHO) classified gastric cancer into distinguish four types<sup>(12)</sup> reasonably simple and used traditional terms, and hence it has neither epidemiologic nor prognostic value. It has not been widely employed. A different approach was elaborated by Lauren<sup>(13)</sup>. We use this classification with our study because it is simple and highly reproducible. It also combined grading with structural features; either intestinal or diffuse. In addition, it has considerable prognostic value: such as the survival rates of patients with the infiltrative type are less than one half of those with the intestinal type. Furthermore, the epidemiology of the intestinal type correlates well with the distribution of chronic atrophic gastritis and, recently, with the prevalence of *H. pylori* infection.

Our results concur with those from a recent European study which found no difference in the occurrence of *H. pylori* between intestinal and diffuse type gastric carcinoma.<sup>(16)</sup> These findings differ from those of an American study in which *H. pylori* was found more frequently in the intestinal type gastric carcinoma than in the diffuse type<sup>(23)</sup>. The reason for this discrepancies is unclear.

In conclusion, our population of patients in this study, forty-five cases of gastric carcinoma at Chulalongkorn hospital in the year 1997, at 95% confidence level, showed no correlation between the histologic type, location of cancer and *H. pylori* infection. The low prevalence of *H. pylori* infection in this study may be genuine, but it

may represent an underestimate for a number of reasons. It indicates that using histology alone is not sufficient for the diagnosis of *H. pylori*. Histological examination can only detect current infection; serology can, in theory, detect both past and present infection. In addition, the sensitivity of histological techniques can be high when specimens should be taken from each site within the stomach to avoid any sampling error. The specificity of histological tests, like the sensitivity, depends on the expertise of pathologist: it can be difficult to identify *H. pylori* when the few bacteria present are of atypical morphology. Special stains were therefore introduced. The Warthin-Starry silver stain, originally used by Warren & Marshall<sup>(24)</sup>, is usually named as the 'gold standard'. This stain is troublesome and expensive. However, the modified Giemsa stain is more practical and is equal or superior to the Warthin-Starry silver stain<sup>(25,26)</sup>. This stain is now widely used, but other stains were also propagated. Specificity, if required, can be achieved using immunocytochemistry with monoclonal or polyclonal preparations. This techniques is not suitable for routine care and does not add a great deal to the existing techniques. Moreover, it is regard to not only the sensitivity and specificity but also the quality of resected specimens. If it is poorly oriented, or is inappropriately fixed or stained, the results will be effected. Nowadays numerous techniques have been proposed for the detection of this organisms. Culture methods are very sensitive and are particularly useful if bacterial numbers are low. Further-



more, the new technique, PCR, is reputed to be extremely sensitive and specific. Most result indicates that the sensitivity of PCR is, in fact, close to that of culture<sup>(27)</sup>. Other possible techniques, the rapid urease test is particularly suited to the detection of *H. pylori*. Sensitivity and specificity are linked, and depend on the lapse before the results of the test are read. Additionally, the urea breath test (UBT), like the rapid urease test, is particularly suited to the detection of *H. pylori*. Sensitivity and specificity depend on the chosen cut-off point. However, the existence of a large number of techniques points that none of them are perfect for every situation. Therefore, it should be recommended for the diagnosis of *H. pylori*, culture and histology should be supplemented with one of the following validated techniques e.g. PCR, UBT, antibody detection etc.. A further limitation on the use of histology arises because the retrospective specimens have been submitted to several pathologists then the standard of sampling site cannot be controlled.

The role of *H. pylori* infection in the pathogenesis of gastric cancer is still an important but unsolved issue. It needs to be further explored.

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