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Histologic Identification of *Helicobacter pylori* and Gastric Pathology in Endoscopic Biopsy Specimens: A Report of 213 cases

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Abstract : **Histology of gastric biopsy specimens from 213 patients was studied. Microscopic observation of hematoxylin & eosin stained sections was the main method for identification of *Helicobacter pylori*. Results of 200 cases were compared with biochemical test, Gram's stain and culture of concurrent specimens. Relations between presence of *H. pylori* and gastric pathology were analyzed. Histologic presence of *H. pylori* is significantly correlated with presence and activity of gastritis and intestinal metaplasia.**

เรื่องย่อ : **เชื้อเฮลิโคแบคเทอร์ พัยลอริ และพยาธิสภาพของชิ้นเนื้อที่ตัดจากกระเพาะอาหารโดยใช้กล้องส่องตรวจ: รายงานผู้ป่วย 213 ราย**

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การศึกษาพยาธิสภาพของชิ้นเนื้อที่ตัดจากการะเพาะอาหารด้วยกล้องส่องตรวจโดยการย้อมสีฮีมาท็อกซีลิน-อีโอซิน เพื่อแสดงเชื้อเฮลิโคแบคเทอร์ พัยลอริ ในผู้ป่วย 213 ราย ในจำนวนนี้มี 200 ราย เปรียบเทียบผลกับวิธีการตรวจทางชีวเคมี, การย้อมสีกรัม และการเพาะเชื้อ พบว่าเชื้อเฮลิโคแบคเทอร์ พัยลอริ มีความสัมพันธ์อย่างมีนัยสำคัญกับการอักเสบ, ความรุนแรงของการอักเสบของกระเพาะอาหาร และการกลายรูปของเยื่อบุกระเพาะอาหารเป็นเซลล์เยื่อบุลำไส้

INTRODUCTION

With the increasing significance of establishing clinical evidence of *H. pylori* infection, the benefit of endoscopy and biopsy for histologic identification has been accepted in clinical practice. This study explored the value of histologic findings, which in addition to the confirmation of the presence or absence of *H. pylori*, furnished the clinician with diagnosis, and description of pathology unavailable otherwise. Histologic identification of *H. pylori* in gastric specimens was systematically recorded and compared with results of identification by biochemical test, Gram's stain, and culture of concurrent fresh specimens. Morphologic parameters in relation to *H. pylori* were analyzed.

MATERIALS AND METHODS

Specimen collection

Gastric and duodenal biopsy specimens were obtained from 213 patients. All biopsies were performed in Ratchaburi Hospital in the years 1991 and 1992. Inclusion criterion in all cases was clinical dyspepsia. Exclusion criteria were recent gastrointestinal hemorrhage and previous gastric surgery. In the analysis, the total number of patients may vary in different tables, as certain parameters were not present in all cases.

All biopsy specimens were immediately fixed in 10% formaldehyde after biopsy, then submitted to the anatomical pathology department. Sections were stained with hematoxylin & eosin.

Bacterial culture

In the same biopsy setting in 200 cases (Table 1), concurrent separate biopsy pieces were submitted to the clinical pathology laboratory for *H. pylori* identification by urease test, Gram's stain and culture in brain heart infusion agar or modified Thayer-Martin medium.[1]

Histopathology

Histologic findings in each of the 213 cases were recorded using a semi-tabulated form designed specifically for this study. Gastritis data were recorded, together with the site of biopsy and presence or absence of ulcer. Presence or absence of *H. pylori*, inflammation, activity of inflammation, atrophy and intestinal metaplasia were graded into 4 degrees (negative or normal, mild, moderate and severe or marked).

Histologic criteria were based on the updated Sydney system of classification and grading of gastritis (Houston Gastritis Workshop 1994).[2,3]

Statistical analysis

The applied statistical tools were percentage, chi-square and Pearson's product moment correlation.

RESULTS

About sixty percent (60.5%) of the 200 cases with histologic and laboratory data were male. Most cases were aged between 30 and 60 years (57.0%) as shown in table 1.

Table 1. Demographic data of patients (n = 200)

Parameters	Number of cases	Percentage
Sex		
Male	121	60.5
Female	79	39.5
Age		
< 30 years	27	13.5
30-60 years	114	57.0
> 60 years	59	29.5

Histologic identification of *H. pylori* was compared to results of three laboratory methods in table 2. The nearest match with histology was the urease test. Diagnostic value of *H. pylori* identification by histology compared to urease test and culture was seen in table 3. Sensitivity and negative predictive value of histology was most closely related to results of urease test. Positive predictive value was highest when compared with Gram's stain.

Table 2. Histologic identification of *H. pylori* compared with urease test, Gram's stain and culture (n = 200)

Histologic identification	Urease		Gram's stain		Culture	
	(+)	(-)	(+)	(-)	(+)	(-)
Positive	83	9	86	6	72	20
Negative	19	89	21	87	26	82
Total	102	98	107	93	98	102

Table 3. Diagnostic value of *H. pylori* by biopsy with urease test and culture as gold standard (n = 200)

Diagnostic predictive value	Gold standard	
	Ureast test	Culture
Sensitivity	81.0 %	74.0 %
Specificity	91.0 %	80.0 %
Accuracy	86.0 %	77.0 %
Positive predictive value	91.2 %	79.1 %
Negative predictive value	82.3 %	76.2 %
Post-test likelihood if test positive	41.5 %	36.0 %
Post-test likelihood if test negative	45.0 %	41.5 %

Accuracy and prevalence of *H. pylori* by histology were highest when compared with urease test. Post-test likelihood if test negative was lowest when compared with urease.

Data of *H. pylori* identification by histology in relation to demographic parameters were shown in table 4. The total number of cases here was 213. Age and sex of patients have no significant correlation with *H. pylori* identification.

The number of biopsy pieces obtained did not show significant relationship with yield of *H. pylori* identification by histology (Table 5).

Table 4. Presence of *H. pylori* in relation to demographic factors (n = 213)

Associated factors	*H. pylori*				p - value
	Positive cases	%	Negative cases	%	
Age (Mean = 49.88 years, SD = 16.45)					
< 30 years (n = 30)	12	40.0	18	60.0	.709
30 – 60 years (n = 122)	59	48.4	63	51.6	
> 60 years (n = 61)	29	47.5	32	52.5	
Total = 213 cases	100	46.9	113	53.1	
Sex					
Male (n = 127)	62	48.8	65	51.2	.509
Female (n = 86)	38	44.2	48	55.8	
Total = 213 cases	100	46.9	113	53.1	

Table 5. Relationship between number of biopsy pieces and presence of *H. pylori* (n = 195)

Number of biopsy pieces	*H. pylori*				p - value
	Positive cases	%	Negative cases	%	
1	74	45.4	89	54.6	.79
2	14	51.9	13	48.1	
3-4	2	40.0	3	60.0	
Total = 195 cases	90	46.2	105	53.8	

As far as activity of gastritis was concerned, *H. pylori* was most often found in moderate gastritis. Of the 91 cases with moderate gastritis, 52 were *H. pylori* positive (57.1%) as shown in table 6.

Table 6. Presence of *H. pylori* and degree of gastritis

Chronic gastritis		*H. pylori* finding			
		Positive	%	Negative	%
None	(n = 35)	1	2.9	34	97.1
Mild	(n = 54)	17	31.5	37	68.5
Moderate	(n = 91)	52	57.1	39	42.9
Marked	(n = 28)	25	89.3	3	10.7
Total	(n = 208)	95	45.7	113	54.3

The crucial information was demonstrated in table 7. *H. pylori* was found to have significant correlation with parameters of inflammation. Histology of chronic gastritis and activity were positively correlated with histologic identification of *H. pylori* at the level of $p<0.01$. Intestinal metaplasia was not significantly correlated at $p<0.05$. Atrophic change had no significance correlation with presence of *H. pylori*.

Table 7. Inflammation associated changes seen in cases with presence of *H. pylori* (n = 213)

Histologic findings	Correlation (r) with *H. pylori*	p - value
Chronic gastritis	.482 **	<0.001
Activity	.485 **	<0.001
Atrophy	.095	.179
Intestinal metaplasia	.018	.800

**p - value < 0.01

H. pylori was found to be present with coexisting ulcer in 16 cases. Of these, the ulcers of ten patients (62.5%) were located in the duodenum (Table 8). Other ulcer sites were the pylorus and channel (12.5%), incisura angularis (12.5%), antrum (6.25%) and the esophagus (6.25%). The relationship between *H. pylori* finding and duodenal ulcer was not statistically significant.

Table 8. Distribution of ulcers found with antral *H. pylori* infection (n = 16)

Ulcer location	Number of cases	%
Duodenum	10	62.5
Pylorus, channel	2	12.5
Incisura	2	12.5
Antrum	1	6.25
Esophagus	1	6.25
Total	16	100

Tables 9 and 10 show the relationship between presence of *H. pylori* and atypia. *H. pylori* was found in 51.8% of cases with these changes. The common epithelial changes were low grade dysplasia and intestinal metaplasia. Atypical changes were not significantly related to presence of *H. pylori*.

Table 9. Presence of *H. pylori* and cellular atypia

Histologic changes		*H. pylori* finding			
		Positive	%	Negative	%
Non-atypia	(n = 149)	68	45.6	81	54.4
Intestinal metaplasia	(n = 24)	9	37.5	15	62.5
Low grade dysplasia	(n = 22)	14	63.6	8	36.4
High grade dysplasia	(n = 9)	5	55.6	4	44.4
Adenocarcinoma	(n = 1)	1	100	-	-
Total	(n = 205)	97	47.3	108	52.7

Table 10. Correlation of *H. pylori* with cellular atypia (n = 205)

Cellular atypia		*H. pylori*				Chi^2	p - value
		Positive cases	%	Negative cases	%		
Positive	(n = 56)	29	51.8	27	48.2	.62	.44
Negative	(n = 149)	68	45.6	81	54.4		
Total	(n = 205)	97	47.3	108	52.7		

DISCUSSION

This series of patients tentatively represented the dyspeptic population in Western Thailand, as the subjects were patients of Ratchaburi Hospital, a medical centre in the west. Other studies explored populations in the capital[4,5], the Northeast[6], and the South[7] of Thailand.

Types of gastritis were not determinable in this series as most biopsies consisted of only one or two pieces and most were from the antrum.

The *H. pylori* infected patients in this study were mostly between 30 and 60 years of age. This was not to be interpreted as the *H. pylori* prevalent age group. It is rather the age group in which clinical dyspepsia is common.[8] There is no statistically significant difference in prevalence of *H. pylori* according to sex or age.[9]

Identification of *H. pylori* by histology was mostly correlated well with urease test. However, false positive cases by histology were present, as other bacteria such as *Helicobacter heilmannii* can be microscopically mistaken for helicobacter pylori. Discrepancies were more apparent when compared with culture specimens. This could be caused by such factors as multifocal distribution of the organism[13], and the technique of specimen handling which could affect culture yield.[10] In some cases, *H. pylori* was negative by histology while urease test was positive. Such results suggested that many factors were operative, such as experience of the pathologist in

interpretation of organism, site of biopsy, amount of the tissue biopsied and multifocal distribution of organisms in the specimens. They also show that histology could demonstrate organisms typical of *H. pylori* infection in a patient with negative urease test.

However, culture is considered the gold standard in identification of *H. pylori*.[10] We recommend the use of all available data for statement of presence or absence of *H. pylori*.

The number of biopsy pieces did not have significant correlation with identification of *H. pylori* in this study. With only one biopsy piece obtained from the antrum in most cases, the data was not adequate for proper statistical analysis. Further studies can be conducted to determine the importance of quantity of biopsy pieces. The fact that parts of the specimens were separated and sent to the clinical laboratory could be related to partial loss of histological information.

While the recommended number and sites of biopsies vary. The minimum should be 5 pieces, two from the antrum, two from the body, and one from the incisura angularis, where atrophic, intestinal metaplastic and dysplastic changes would usually be first observable[2,11,14]. In addition to getting better representation, this would increase the chance of adequacy, as some biopsy pieces could turned out to be superficial and did not include the muscularis mucosae as required. The clinician can add more specimens from suspicious areas.

In this study, *H. pylori* was histologically identified using H&E stained section. The authors believe that attentive observation, although time-consuming at first, is essential. It is recommended that a special-stained section be added to complement H&E stained section as a routine. This may be the Giemsa stain, the Warthin-Starry stain, or the modified toluidine blue stain, which is practical and economical.[10] It has to be added that, however careful the pathologist may be, H. pylori can be absent from a non-representative section.

The data presented here clearly points out that *H. pylori* is significantly correlated with the degree of chronic inflammation, and the degree of activity. When a pathologist finds evidence of gastritis, chronic gastritis, or activity in gastritis, he should look for *H. pylori* infection, and vice versa. Correlation of lesser degree but still significant is between *H. pylori* and intestinal metaplasia.

Activity of gastritis is highly important, as it had been shown in this study that the finding of *H. pylori* correlates best with moderate gastritis.

The relationship between antral *H. pylori* and duodenal peptic ulcer was observed in this study, as most ulcers were found in the duodenum. This was in accordance with studies in Europe and the United States.[12]

The value of histologic examination justifies the effort to biopsy on the part of the endoscopist. The increased cost is minimal and adds to the value of clinical investigation, considering the patient having to undergo the endoscopic procedure itself. The information obtained from histology is irreplaceable by any other *H. pylori* identifying technique. Histology reveals the nature of the gastric disease, and helps to locate, identify and indicate degree of H. pylori infection. Other laboratory techniques are useful in screening and follow-up care of patients with *H. pylori*-related diseases.

The tabulated format of recording histologic parameters and description helped facilitate filing and statistical analysis. A pathologist can customise his form for routine use or for subsequent studies. The format used conformed to the updated Sydney system. The degrees of histologic changes were graded and tabulated, which helped in statistical analysis.

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