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Distribution of *H.pylori* antigens in gastric mucosa and its significance

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Abstract: Objective: To investigate the distribution of *H.pylori* antigens in the gastric mucosa in patients with *H.pylori* infection, and the relationship between the distribution and gastric cancer. Methods: Of 112 patients confirmed by pathological study to have chronic superficial gastritis, precancerous changes (chronic atrophic gastritis, intestinal metaplasia or atypical hyperplasia) and gastric cancer, 28 were *H.pylori* negative and 84 were *H.pylori* positive. *H.pylori* antigens in the gastric mucosa were detected by immunohistochemistry. Results: The *H.pylori* positive group, comprised 12 of 22 (50.0%) in the chronic superficial gastritis group, 22 of 25 (88.0%) in the precancerous changes group and 13 of 35 (37.1%) in the gastric cancer group. The positive rates of *H.pylori* antigens in the cytoplasm progressively increased, respectively at 0.0% (0/12), 63.6% (14/22) and 84.6% (11/13) for the same groups (χ^2=19.76, P=0.000); *H.pylori* antigens were located in the mucus layer and above the neck of the mucosal gland in 9 of 12 (75.0%) cases with chronic superficial gastritis, at the neck of the mucosal gland and the isthmus in 12 of 22 (54.5%) cases with precancerous changes, below the isthmus in 9 of 13 (69.2%) cases with gastric cancer (χ^2=25.30, P=0.000). In the *H.pylori* negative group, no *H.pylori* antigen was observed. Conclusion: With the progression of chronic superficial gastritis→precancerous changes→gastric cancer, *H.pylori* antigens progressively migrated from the outer part to the inner part of the cell, and from the superficial to the deep gastric mucosa.

Key words: *Helicobacter pylori*, Immunohistochemistry, Antigen, Gastric cancer, Precancerous change
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INTRODUCTION

Helicobacter pylori (*H.pylori*) was grouped as a class I carcinogen by the International Agency for Research on Cancer in 1994. A direct relation between *H.pylori* infection and gastric carcinogenesis was demonstrated in 1998 in an experimental animal model (Watanabe *et al.*, 1998). However, the role of *H.pylori* in human gastric carcinogenesis is supported almost exclusively by epidemiological data and prospective histopathological studies. So far the mechanism of *H.pylori* carcinogenesis has not been clarified yet. *H.pylori* usually lies in the mucus layer, gastric pits, and above the epithelial cells. However, transmission electron microscopy showed *H.pylori* was engulfed and degraded by human gastric cancer cell line SGC-7901 (Yang *et al.*, 2000). Electron or immunoelectron microscopy showed *H.pylori* destroys the junction of cells, and invades the gastric mucosa (Yu *et al.*, 1997; Ko *et al.*, 1999). These findings indicated the invasiveness of *H.pylori* was important in the pathogenesis of gastric cancer. *H.pylori* was shown by immunohistochemistry to invade the cytoplasm of epithelial cells (Engstrand *et al.*, 1997). The relationship between the distribution of *H.pylori* antigens and gastric cancer has not been previously elucidated. The aim of our study is to investigate the distribution of *H.pylori* antigens in

the gastric mucosa of patients with gastric cancer.

MATERIALS AND METHODS

Clinical data

Use of *H.pylori* diagnostic criteria showed that out of 112 patients, 28 were *H.pylori* negative and 84 were *H.pylori* positive. Among the *H.pylori* positive group, there were 24 cases with chronic superficial gastritis, 25 cases with precancerous changes (chronic atrophic gastritis, intestinal metaplasia or atypical hyperplasia) and 35 cases with gastric cancer, and among the *H.pylori* negative group were 10, 16 and 2 cases of gastric cancer.

Tests of *H.pylori* infection

Histological evaluation was performed with haematoxylin and eosin stain and the *H.pylori* methylene blue staining kit (Fujian Sanqiang, China). Rapid urease tests (Digestech, China), ^{14}C-urea breath tests (Shenzhen Headway, China), and serological *H.pylori*-IgG tests (Orion Diagnostica, Finland) were carried out as suggested by each manufacturer. The diagnostic criteria for *H.pylori* infection were referred to consensus of the Chinese Society of Gastroenterology in 1999. Patients were considered negative for *H.pylori* infection if the four tests were all negative.

Immunohistochemical technique

After deparaffination and rehydration of the specimens, *H.pylori* antigen was detected by using the Utrasensitive SAP kit (Maxim Biotech, USA). The first antibody was rabbit anti *Helicobacter pylori* (DAKO, Denmark). The sections were counterstained with Nuclear Fast Red (Maxim Biotech, USA) and examined under a light microscope.

RESULTS

Positive rates of *H.pylori* antigens in the epithelial cell cytoplasm (Table 1)

The *H.pylori* positive group comprised 12 of 22 (50.0%) in the chronic superficial gastritis group, 22 of 25 (88.0%) in the precancerous changes group and 13 of 35 (37.1%) in the gastric cancer group. The positive rates of *H.pylori* antigens in the cytoplasm progressively increased, respectively at 0.0% (0/12), 63.6% (14/22) and 84.6% (11/13) for the same groups (χ^2=19.76, P=0.000). In the *H.pylori* negative group, no *H.pylori* antigen was observed.

Table 1 Positive rates of *H.pylori* antigens in the epithelial cell cytoplasm

Group		N	Antigen positive number	Positive rates of cytoplasm
H.pylori positive group	CSG	24	12	0.0%(0/12)*
	Precancerous changes	25	22	63.6%(14/22)*
	GC	35	13	84.6%(11/13)*
H.pylori negative group		28	0	0.0%(0/ 0)

Pearson Chi-Square: *χ^2=19.76, P=0.000;
CSG: chronic superficial gastritis; GC: gastric cancer

Distribution of *H.pylori* antigens in the gastric mucosa (Table 2)

In the 12 chronic superficial gastritis cases, *H.pylori* antigens were located superficially in the mucus layer and above the neck of the mucosal gland in 9 (75.0%) cases, at the neck of the mucosal gland and the isthmus in 3 (25.0%) cases; In the 22 patients with precancerous changes, *H.pylori* antigens were located in the mucus layer and above the neck of the mucosal gland in 5 (22.7%) cases, at the neck of the mucosal gland and the isthmus in 12 (54.5%) cases, below the isthmus in 5 (22.7%) cases;

Table 2 Distribution of *H.pylori* antigens in the gastric mucosa

H.pylori positive group	Antigen positive number	Distribution of *H.pylori* antigens*		
		Mucus layer and above the neck of the mucosal gland	Neck of the mucosal gland and isthmus	Below the isthmus
CSG	12	75.0%(9)	25.0%(3)	0.0%(0)
Precancerous changes	22	22.7%(5)	54.5%(12)	22.7%(5)
GC	13	0.0%(0)	30.8%(4)	69.2%(9)

Pearson Chi-Square: *χ^2 = 25.30, P = 0.000; CSG: chronic superficial gastritis; GC: gastric cancer

in the 13 gastric cancer cases, *H.pylori* antigens were located at the neck of the mucosal gland and the isthmus in 4 (30.8%) cases, and below the isthmus in 9 (69.2%) cases (χ^2=25.30, P=0.000).

DISCUSSION

The relationship between *H.pylori* and gastric cancer had been established (Watanabe *et al.*, 1998), but the mechanism has not been clarified yet. The invasiveness of *H.pylori* has recently aroused interest. Our study showed the positive rates of *H.pylori* antigens in the cytoplasm progressively increased with increasing malignancy. This indicated that *H.pylori* antigens progressively migrated from the cell membrane to the inner cytoplasm with the progression of chronic superficial gastritis→precancerous changes→gastric cancer; and that this change was associated with *H.pylori* carcinogenesis. *H.pylori* usually lies in the mucus layer, gastric pits and above the epithelial cells, but recently its depth of invasion has been considered significant. *H.pylori* could destroy the cell junctions and invade the gastric mucosa, or be engulfed and degraded by epithelial cells (Yang *et al.*, 2000; Yu *et al.*, 1997; Ko *et al.*, 1999). Invasion by *H.pylori* antigens involved absorption of soluble *H.pylori* products, engulfment by epithelial cells and transport through damaged tight junctions. *H.pylori* was shown by immunohistochemistry to invade the cytoplasm of the epithelial cell in ten healthy volunteers with asymptomatic *H.pylori* gastritis (Engstrand *et al.*, 1997). There were few *H.pylori* antigens in gastric cancer tissue, because the microenvironment of cancer tissue was not favorable for bacterial growth. Under hardship conditions, *H.pylori* morphology changes from spiral to coccoid forms; and part of *H.pylori* coccoid forms may be viable (Zheng *et al.*, 1999). Immunohistochemical staining and electron microscopy revealed *H.pylori* L-forms in the cytoplasm of malignant cells in 91 of 136 cases with gastric neoplasm (Yu *et al.*, 1997). Thus, *H.pylori* possesses the capacity of invasiveness, and some forms of *H.pylori* that invade in the epithelial cells may be associated with gastric malignancy.

In our study, *H.pylori* antigens were located in the mucus layer and above the neck of the mucosal gland in 9 of 12 (75.0%) cases with chronic superficial gastritis, at the neck of the mucosal gland and the isthmus in 12 of 22 (54.5%) cases with precancerous changes; below the isthmus in 9 of 13 (69.2%) cases with gastric cancer. These results indicated an association between the migration of *H.pylori* antigens and gastric malignancy. Undifferentiated cells in the neck of the mucosal gland constitute the proliferation center of gastric mucosa, and replace necrotic epithelial cells by division and proliferation. *H.pylori* antigens which invade the gastric mucosa, may activate neutrophils and T cells which may produce excessive oxygen-derived free radicals. Base modification and rupture of DNA chains in nucleic acid are the results of attack by the radicals, and play an important role in the carcinogenesis (Ernst, 1999; Davies *et al.*, 1994; Cerutti, 1994). Cells in the mitotic phase are more damageable than in the quiescent period. Most *H.pylori* antigens lay at the neck of the mucosal gland and the isthmus in cases with precancerous changes. While undifferentiated cells do not renovate and fall off, once the genetic alteration and the mutations occur, neoplastic transformation is likely. Thus, when *H.pylori* invades the neck of the mucosal gland and the isthmus, it may represent an important step towards carcinogenesis. In neoplastic tissue, uncontrolled proliferation results in structural damage which may allow *H.pylori* antigens to penetrate the deep mucosa.

In conclusion, *H.pylori* possesses the capacity of invasiveness, and some forms of *H.pylori* which penetrate into the epithelial cells, may be associated with gastric malignancy. With the progression of chronic superficial gastritis→precancerous lesions →gastric cancer, *H.pylori* antigens progressively migrated from the superficial mucosa to the deep mucosa, and from the cell membrane to the inner cytoplasm.

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