Effect of *Helicobacter pylori* infection on gastric mucosal pathologic change and level of nitric oxide and nitric oxide synthase

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**INTRODUCTION**

There is evidence that *Helicobacter pylori* (*H. pylori*) is closely related with gastric carcinoma, and is considered as the first grade oncogene of gastric carcinoma by World Health Organization (WHO). *H. pylori* infection correlates closely with gastric mucous pathology\[7-11\].

NO is a medium produced in vessel endothelial cells or smooth muscle cells by NOS\[12\]. As an inflammatory medium, NO plays an important role in the physical function and pathological process. Changes of NO in serum and tissue are related with damage to gastric mucosa and *H. pylori* infection\[10-11\].

This study aimed to investigate the changes of NO, NOS and the pathological transformation of gastric mucosa in patients infected with *H. pylori*.

**MATERIALS AND METHODS**

**Patients**

Two hundred and eighty-two patients with chronic gastric disease were enrolled in this study. *H. pylori* was detected by both rapid urease test and real-time fluorescent quantitative PCR in these patients. Anti-CagA-IgG was detected in the *H. pylori* positive patients, the serum samples were collected from 50 *H. pylori* positive patients and 35 *H. pylori* negative patients for detection of NO and NOS.

**Real-time fluorescent quantitative PCR**

Real-time fluorescent quantitative PCR was performed with PCR kit (Da’an Gene Diagnosis Center, Guangzhou). Fluorescence was detected with a type DA620 fluorescent detector.

**CagA H pylori-IgG**

CagA *H. pylori* IgG was detected according to the manufacturer’s instructions (Shanghai Jingying Biology Corporation).

**Measurement of NO and NOS**

Because NO could be converted into NO\(_2\) and NO\(_3\) in *vivo*, nitrate reductase was used to deoxidize NO\(_3\) into NO\(_2\), and to determine its concentration. NO and NOS were tested with the kits, (Nanjing Jiancheng Biology Corporation).

**Statistical analysis**

Data were presented as mean±SD and analyzed with SPSS software. Statistical analysis was performed using two-tailed Student’s *t* test and *χ*\(^2\) test. *P*<0.05 was considered statistically significant.
RESULTS

Relationship between H pylori infection and pathology
Among the 282 cases, H pylori was found in 150 cases, (53.19%), including 38.54% (37/96) in chronic superficial gastritis group, 51.26% (61/119) in atrophic gastritis group, 73.17% (30/41) in intestinal metaplasia group, and 84.62% (22/26) in dysplasia group. The H pylori positive rate in atrophic gastritis group was higher than that in chronic superficial gastritis group (P<0.05), and significantly higher in intestinal metaplasia group and dysplasia group than that in chronic superficial gastritis group (P<0.01, Table 1).

| Group       | n   | H pylori positive | H pylori negative |
|-------------|-----|------------------|------------------|
| CSG         | 16  | 80.0±14.6        | 60.0±16.4        |
| CAG         | 25  | 95.4±18.4        | 74.6±19.2        |
| IM          | 12  | 91.2±13.9        | 75.5±27.7        |
| Dysplasia   | 9   | 95.3±10.3        | 71.5±19.6        |

(a)P<0.05 vs CSG.

Relationship between anti-CagA-IgG and pathology
The anti-CagA-IgG positive rate was 71.33% (107/150) in 150 H pylori positive patients, including 40.54% (15/37) in chronic superficial gastritis group, 75.41% (46/61) in atrophic gastritis group, 86.67% (26/30) in intestinal metaplasia group and 90.91% (20/22) in dysplasia group. The anti-CagA-IgG positive rate in chronic superficial gastritis group was significantly lower than that in the other three groups (Table 2).

| Group       | n   | Concentration |
|-------------|-----|---------------|
| CSG         | 16  | 80±14.6       |
| CAG         | 25  | 95.4±18.4     |
| IM          | 12  | 91.2±13.9     |
| Dysplasia   | 9   | 95.3±10.3     |

(a)P<0.05 vs CSG.

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(a)P<0.05 vs CSG.

Relationship between NO, NOS, and pathology
The serum concentration of NO and NOS was 87.6±16.1 µmol/L and 51.4±13.3 µmol/L respectively in H pylori positive group, and 69.8±19.4 µmol/L and 35.2±13.3 µmol/L respectively in H pylori negative group (Table 3).

| Group               | n   | NO(µmol/L) | NOS(µmol/L) |
|---------------------|-----|------------|-------------|
| H pylori positive    | 50  | 87.6±16.1  | 51.4±13.3  |
| H pylori negative    | 35  | 69.8±19.4  | 35.2±13.3  |

(a)P<0.01 vs H pylori negative group.

DISCUSSION

H pylori infection plays a leading role in the pathogenesis of chronic gastritis. Furthermore, H pylori infection is also a high risk factor for the development of gastric cancer[12]. H pylori can destroy gastric mucosa, leading to inflammation of gastric mucosa and digestive symptoms.

Our study showed that the H pylori positive rate in chronic superficial gastritis group was 38.54%, suggesting that H pylori is related to inflammation of gastric mucosa. Other factors may be involved in inflammation of gastric mucosa, such as pH value, mucus, glycoprotein. But in atrophic gastritis group, intestinal metaplasia group, and dysplasia group, the H pylori positive rate was 51.26%, 73.17% and 84.62%, respectively, indicating that H pylori infection has a close relationship with gastric pre-neoplastic diseases, such as atrophy, intestinal metaplasia, and dysplasia.

It was reported that H pylori has two types. Type I H pylori possesses high virulence energy producing cytotoxin-associated protein A and vacuole toxin, which are responsible for inflammatory response of gastric epithelial cells, and promotes cell proliferation and apoptosis[13,14]. Therefore, type I H pylori has a close relationship with development of gastric pre-neoplastic diseases[15-18]. Our study showed that
the pathological change of gastric mucosa was parallel with the anti-CagA-IgG positive rate. These observations support the hypothesis that type I H. pylori infection is a high risk factor for the development of gastric pre-neoplastic diseases.

It has been proved that there are lots of NOS in smooth muscle cells and myenteric nerve plexus of stomach, which are induced to produce endogenic NO by cytotoxins of H. pylori. Moreover, a high pH value is beneficial for anaerobes to colonize in the stomach, and can degrade nitrate of food into nitrite. NO is regarded as an important inflammatory medium, related with acute and chronic inflammatory responses. But NO seems to have both beneficial and harmful effects on different stages of inflammation. In earlier period, NO can relieve mucosal inflammation and prevents cellular damage. However, it can prevent cellular apoptosis, induce mutation and contribute to the development of gastric pre-neoplastic diseases in later period.

In this study, the levels of NO and NOS in chronic superficial gastritis group were significantly lower than those in pre-neoplastic diseases groups, such as atrophic gastritis, superficial gastritis group. However, the levels of NO in pre-neoplastic diseases groups, such as atrophic gastritis, superficial gastritis group were significantly lower than those in other pathological groups, suggesting that the serum level of NO induced by H. pylori may be related with pre-neoplastic diseases. In H. pylori negative patients, the levels of NO in NO and NOS level in every pathological group, but the levels of NO were significantly higher in gastric pre-neoplastic disease groups, showing that other ways may stimulate the producing of NO besides H. pylori in pre-neoplastic diseases. However, we believe that NO plays an important role in the development of pre-neoplastic diseases.

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