Gastrointestinal Cancer

Helicobacter pylori Infection in Association with Cell Proliferation，Apoptosis and Prostaglandin E_2 Levels

PAN Kai-feng (潘凯枫), ZHANG Yang (张阳), ZHANG Lian (张联), MA Jun-ling (马峻岭)
FENG Guo-shuang (冯国双), ZHOU Tong (周彤), YOU Wei-cheng (游伟程)*

Department of Cancer Epidemiology, Beijing University School of Oncology, Beijing Institute for Cancer Research, Beijing Cancer Hospital, Beijing 100036

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ABSTRACT

Objective: To evaluate the relationship between *H. pylori* infection with cell proliferation, apoptosis and PGE_2 levels. **Methods:** A population-based study was conducted in Linqu, a high-risk area of gastric cancer in China. A total of 1523 subjects, aged 35-64, participating in a gastric cancer screening survey were investigated. *H. pylori* status were determined by ^{13}C-urea breath test, expressions of Ki-67 were assessed by immunohistochemistry, apoptotic cells were detected by terminal deoxynucleotide transferase mediated dUTP nick end-labeling (TUNEL) method, and PGE_2 levels were measured by enzyme immunoassay. **Results:** *H. pylori* infection was positively associated with cell proliferation activity. The mean and median percentage of Ki-67 labeling index (LI) in subjects with *H. pylori* positive were 14.1±10.3 and 12.0, significantly higher than those with *H. pylori* negative ($\bar{x}\pm s$: 8.4±7.0; median: 5.8; $P<0.0001$). Moreover, the prevalence rates of *H. pylori* infection showed a tendency to increase according to severity score of cell apoptosis ($P_{trend}<0.0001$), from score 0 to 3, the percentage of *H. pylori* positivity increased from 67.5% to 96.7%. Furthermore, The mean and median of PGE_2 concentration were 628.84±726.40 pg/mL and 411.33 pg/mL among subjects with *H. pylori* positive compared with 658.19±575.91pg/mL and 455.97 pg/mL among those with *H. pylori* negative ($P=0.209$). **Conclusion:** *H. pylori* infection was positively associated with increased cell proliferation and apoptosis activity, suggesting that *H. pylori* infection plays an important role in the gastric epithelial cell malignant transformation.

Key words: Helicobacter pylori; Gastric cancer; Cell proliferation; Apoptosis; PGE2

Gastric cancer (GC) is the second leading cause of cancer mortality in China[1,2]. Linqu County, a rural area in Shandong Province of northeast China, has one of the highest rates of gastric cancer in the world (age-adjusted rate exceeding 70 deaths per 100,000 males)[3]. The prevalence of precancerous gastric lesions is very high, and the risk of GC is increased significantly with the severity of the gastric lesions in Linqu[4].

Although the cause and biological mechanism underlying gastric carcinogenesis are not fully understood in Linqu, our previous studies in Linqu revealed that *H. pylori* infection played a crucial role in the development of GC[5,6]. A randomized intervention trial indicated that the eradication of *H. pylori* reduced the risk by 40% in the prevalence of severe chronic atrophic gastritis (CAG), intestinal metaplasia (IM), dysplasia (DYS) or GC[7].

Many molecular events are involved in the *H. pylori*-associated process of gastric carcinogenesis. *H. pylori* infection can induce host inflammatory and immune responses, releasing the cytokines and

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***Author** to whom correspondence should be addressed.
E-mail: weichengyou@yahoo.com

stimulating cell proliferation and apoptosis[8]. Moreover, *H. pylori* infection could induce cyclooxygenase (COX)-2 up-regulations[8,9]. COX-2, the enzyme that catalyses the conversion of arachidonic acid to prostaglandins (PGs), could stimulate tumor cell proliferation, inhibit apoptosis and increase the invasiveness of malignant cells[10]. Clinical studies with small cases have shown that an increased cell proliferation and apoptosis was observed in *H. pylori*-associated gastritis[11,12], and *H. pylori* infection was positively associated with PGE_2 levels[8,13].

H. pylori infection plays an important role in gastric carcinogenesis. However, its role on the gastric epithelial cell turnover and association with prostaglandin E2 (PGE2) levels has not been fully established. In the present study, we evaluated the association between *H. pylori* infection with cell proliferation, apoptosis and PGE_2 levels in a high-risk population in Linqu.

MATERIALS AND METHODS

Study Population

In 2002, a gastric endoscopic screening survey was conducted in 12 villages in Linqu County. A total of 2813 subjects, representing 80% of eligible residents aged 35-64 years, received an endoscopic examination and a ^{13}C-urea breath test (^{13}C-UBT). Each subject was interviewed using a structured questionnaire to obtain information on historical cigarette smoking, alcohol consumption.

The detailed procedures have been described elsewhere[3]. Briefly, the gastric mucosa was examined and five biopsies were obtained from standard sites of the stomach according to the Updated Sydney System[14]. All specimens were reviewed by a panel of three pathologists according to the Updated Sydney System and Padova International Classification[14,15]. Each biopsy was assigned a global diagnosis based upon the most severe diagnosis among any of the five biopsies.

For the current study, a total of 1523 subjects were selected at random with a spectrum of gastric pathology. In addition, a total of 310 subjects were selected from 1523 subjects to determine PGE_2 levels. One or two additional biopsies were obtained from the lesser curvature of antrum or angulus adjacent the standard sites in 310 subjects and frozen in liquid nitrogen immediately. A written informed consent was obtained from each participant and the study was approved by the Institutional Review Board of Peking University School of Oncology.

^{13}C-Urea Breath Test

The ^{13}C-UBT was performed as previously reported[16]. Briefly, after a baseline breath sample had been taken, fasting patients were given 80 mg^{13} C-urea (>99%) in 100 ml water. An additional breath sample was taken at 30 min after urea administration. Breath samples obtained at baseline and after drug administration were analyzed for $^{13}CO_2$ with a gas isotopic ratio mass spectrometer (GIRMS), and any concentration of $^{13}CO_2$ at 30 min that exceeded the baseline concentration more than 4 parts per 1000 (> 0.4%) was regarded as a positive result. Samples of duplication were tested with an agreement of 97.2%.

Immunohistochemical Analysis

Expression of Ki-67 was detected by immunohistochemical analysis. Tissue specimens were fixed in 10% neutral-buffered formalin and were paraffin embedded according to standard procedures. The tissue blocks were cut into 4-μm sections and mounted onto slides, the specimens were deparaffinized and rehydrated, and treated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. Antigen retrieval was performed by microwave for 7min. A monoclonal mouse antibody to Ki-67 (diluted 1:100; Zhongshan Golden Bridge Co.) was used as primary antibodies at 4℃ overnight. Anti-mouse immunoglobulin G (GBI) labeled with biotin was used as a secondary antibody and slides were incubated for 30 min at 37 ℃, followed by incubation with peroxidase-conjugated avidin. Peroxidase was visualized by use of the 3,3'-diaminobenzidine substrate (DAB) kit (Zhongshan Golden Bridge Co.). Negative controls were done by omitting the primary antibody.

The Ki-67 labeling index (LI) was determined by observing 500 cell nuclei in areas of the section with highest labeling frequency, and the percentage of Ki-67 labeled nuclei was used for analysis.

In Situ Labeling for Apoptosis

In situ detection of apoptotic cell was performed by terminal deoxynucleotide transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) using a commercial kit (TACS.XL™-Basic, R&D Systems, Inc.), and following manufacture's instructions. Briefly, after deparaffinization and rehydration, tissue sections were incubated