Epidermal growth factor and prostaglandin E2 levels in *Helicobacter pylori*-positive gastric intraepithelial neoplasia

Zhitao Chen, Jie Wu, Dan Xu, Manling Huang, Shengbin Sun, Heng Zhang, Xiaodong Huang and Ping Wang

Abstract

Objective: To investigate levels of epidermal growth factor (EGF) and prostaglandin E2 (PGE2) in Han Chinese patients with *Helicobacter pylori*-positive gastric low-grade intraepithelial neoplasia (LGIN).

Methods: In this prospective, observational study, gastric specimens from patients with LGIN were collected by gastroscopy with consecutive biopsy. EGF and PGE2 concentrations in serum and gastric juice from patients with LGIN were measured by enzyme-linked immunosorbent assay. Presence of *H. pylori* infection was assessed in patients with LGIN and healthy controls.

Results: Out of 5638 patients and 548 controls, *H. pylori* infection in patients with chronic gastritis was associated with disease type (endoscopic classification) and disease severity. Patients with *H. pylori*-positive LGIN had significantly higher concentrations of serum EGF and lower concentrations of serum PGE2 versus patients with *H. pylori*-negative LGIN. Serum EGF and PGE2 levels in patients with LGIN were not significantly associated with disease type, but were significantly associated with disease severity.

Conclusions: *H. pylori* infection was associated with chronic gastritis type (endoscopic classification) and disease severity. Abnormal EGF and PGE2 levels may be associated with *H. pylori*-positive LGIN in Han Chinese patients in central China.

Keywords

Chronic gastritis, gastric intraepithelial neoplasia, Helicobacter pylori, prostaglandin E2, epidermal growth factor

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Corresponding author:
Jie Wu, Department of Gastroenterology and Central Laboratory, Central Hospital of Wuhan, 26 Sheng Li Street, Wuhan 430014, Hubei Province, China.
Email: wujie_20120803@163.com
Introduction

Precancerous lesions are histological abnormalities that may be associated with an increased risk of developing cancer, and according to the World Health Organization (WHO) classification of tumours of the digestive system,\(^1\) the recommended terms for gastric precancerous lesions are low-grade intraepithelial neoplasia (LGIN) and high-grade intraepithelial neoplasia. In the Correa cascade of multistep gastric carcinogenesis, an inflammation-metaplasia-dysplasia-carcinoma sequence indicates that neoplasia may be a critical point for malignant transformation.\(^2\)

The majority of gastric cancers are known to be associated with *Helicobacter pylori* infection, and *H. pylori* is considered to be a single risk factor for human gastric cancers by the International Agency for Research on Cancer.\(^3\) The chain of events leading to gastric cancer associated with *H. pylori* begins with normal gastric mucosa, followed by chronic superficial gastritis, atrophic gastritis, intestinal metaplasia, dysplasia, and finally adenocarcinoma.\(^4\) Atrophic gastritis and intestinal metaplasia are thought to exponentially increase the risk of developing gastric cancer.\(^4\)

Abnormal levels of epidermal growth factor (EGF) and prostaglandin E2 (PGE2) in gastric mucosa may induce changes in gastric epithelial cells that lead to cancer.\(^5,6\) Levels of serum and gastric juice EGF and PGE2 in patients with LGIN remain unclear. Thus, in the present study, prevalence of *H. pylori* infection, and the relationship between *H. pylori* infection and type of chronic gastritis (assessed via gastroscope), and disease severity, were analysed in Han Chinese patients with chronic gastritis in central China. Serum and gastric juice levels of EGF and PGE2 in patients with LGIN were also determined.

Patients and methods

Study population

In this prospective observational study, Chinese patients with chronic gastritis being treated at the Central Hospital of Wuhan, Wuhan, China were sequentially enrolled between May 2011 and April 2013. There were no predefined exclusion criteria. Gastric mucosal biopsy was performed on consecutive patients with chronic gastritis undergoing diagnostic gastroscopy. Endoscopic classification of chronic gastritis was categorized into superficial gastritis, erosive gastritis, haemorrhagic gastritis or atrophic gastritis according to the Dalian Academic Conference criteria of the Chinese Society of Digestive Endoscopy.\(^7\) LGIN was determined according to the WHO Classification of Tumours of the Digestive System.\(^1\) The severity of chronic gastritis was classified according to pathology (mild; moderate; severe).\(^8\)

For analysis of EGF and PGE2 concentrations in serum and gastric juice, a random subgroup of patients with LGIN were selected from the patients with chronic gastritis. Pathological and endoscopic diagnoses of all patients were performed by three independent pathologists and three independent endoscopists from The Central Hospital of Wuhan.

Age- and sex-matched healthy controls were selected from medical staff of The Central Hospital of Wuhan, and from healthy volunteers in the same geographic area of Wuhan city. Healthy controls were ethnically matched, had no symptoms suggestive of chronic digestive tract disease and had no personal or family history of gastric cancer.

The study was approved by the Ethics Committee of The Central Hospital of Wuhan, and written informed consent was obtained from all study participants.
Detection of H. pylori infection

Rapid urease and C\textsuperscript{14} breath tests were performed in patients with chronic gastritis and healthy controls, to investigate the presence of \textit{H. pylori} infection. Participants were considered to be \textit{H. pylori}-positive if both tests gave a positive result.

Rapid urease tests were performed by placing an endoscopic biopsy specimen into a small amount of solution containing urea, phenol red and preservatives. In the presence of \textit{H. pylori}, bacterial urease hydrolysed the urea and produced ammonia, resulting in a yellow-to-red colour change. Results were read between 1 min and 24 h. C\textsuperscript{14} breath tests were performed as follows: Each participant swallowed a capsule of urea labelled with 37 kBq (1 \mu Ci) of C\textsuperscript{14} and after 10 min was asked to exhale into a balloon (Heliprobe BreathCard\textsuperscript{TM}, Kibion, Stockholm, Sweden), to collect a breath sample, until the indicator membrane changed colour from orange to yellow. In the presence of \textit{H. pylori} in the stomach, urea would be converted into C\textsuperscript{14}-labelled carbon dioxide by urease enzyme. Samples with values $\leq$25 counts per min (cpm) were considered negative for \textit{H. pylori}; those with values $\geq$50 cpm were considered positive for \textit{H. pylori} infection.

Determination of EGF and PGE2 concentrations

A total of 4 ml venous blood from each patient with LGIN was collected into serum separator tubes and allowed to clot for 30 min at room temperature, followed by centrifugation for 10 min at 1000 \textit{g} at room temperature. All serum samples were stored at $-70^\circ$C until use.

Gastric juice samples were collected from patients with LGIN into 4 ml centrifuge tubes, and with 20 min following collection, were centrifuged at 2000 \textit{g} for 30 min at room temperature. The supernatant was then separated into a 2 ml RNase-free centrifuge tube and stored at $-70^\circ$C until use.

The EGF and PGE2 concentrations in serum and gastric juice of patients with LGIN were measured by human EGF enzyme-linked immunosorbent assay (ELISA) kit (Cat. No. BMS20701INST, Bender Medsystems, Vienna, Austria) and prostaglandin E2 high sensitivity ELISA Kit (Cat. No. ab133055, Abcam, Cambridge, MA, USA), respectively, according to the manufacturer’s protocols. Plates were read at 450 nm and 405 nm against standard curves for EGF and PGE2, respectively, with detection sensitivity for EGF and PGE2 of 0.26 pg/ml and 8.26 pg/ml, respectively. Each sample was tested in triplicate.

Statistical analyses

Statistical analyses were performed using SPSS\textsuperscript{®} software, version 17.0 (SPSS Inc., Chicago, USA). The sample size was estimated at the 0.05 level of significance with a two-sided test, and 88\% statistical power to detect an effect with a relative risk of 2.0. Between-group comparisons of the \textit{H. pylori} infection rate were performed using \textit{$\chi^2$}-test. Continuous variables were presented as mean $\pm$ SD, and were statistically analysed using two-tailed, Student’s \textit{t}-test and one-way analysis of variance. A \textit{P} value $< 0.05$ was considered statistically significant.

Results

A total of 5,638 Chinese patients with chronic gastritis, comprising 3,219 (57.09\%) male and 2,419 (42.91\%) female patients (mean age, 59.25 $\pm$ 19.58 years), and 548 age- and sex-matched healthy controls (303 [55.29\%] male and 245 [44.71\%] female; mean age, 56.23 $\pm$ 13.45 years) were included in this study. Of the patients with
chronic gastritis, 82 patients with LGIN were randomly selected for analysis of EGF and PGE2 concentrations in serum and gastric juice. Among these patients with LGIN, 49 (59.76%) were male and 33 (40.24%) were female (mean age, 53.38 ± 14.26 years).

Prevalence of H. pylori infection

The prevalence of H. pylori was higher in patients with chronic gastritis compared with healthy controls ($\chi^2 = 285.8$, $P < 0.01$; Table 1), and was higher in patients with erosive gastritis versus superficial gastritis ($\chi^2 = 191.08$, $P < 0.01$; Table 1).

When patients with chronic gastritis were classified according to pathological severity, the prevalence of H. pylori infection was higher in those with severe inflammation than in those with mild or moderate inflammation ($\chi^2 = 234.5$, $P < 0.01$; $\chi^2 = 111.5$, $P < 0.01$, respectively; Table 2).

In patients with chronic gastritis, the prevalence of H. pylori infection was higher in patients with LGIN than in patients with non-LGIN (264/294 [89.80%] versus 3042/5344 [56.92%], $\chi^2 = 122.8$, $P < 0.01$).

Serum and gastric juice EGF and PGE2 concentrations in patients with LGIN

Patients with H. pylori-positive LGIN ($n = 58$) had significantly higher concentrations of serum EGF (180.67 ± 49.93 ng/l versus 68.51 ± 1.74 ng/l, respectively; $P < 0.01$), and lower concentrations of serum PGE2 (11.80 ± 1.83 ng/l versus 20.95 ± 3.40 ng/l, respectively; $P < 0.01$) than patients with H. pylori-negative LGIN ($n = 24$). In patients with LGIN, gastric juice EGF and PGE2 concentrations were not significantly different between H. pylori-positive patients and H. pylori-negative patients (EGF, 70.67 ± 15.21 ng/l versus 56.78 ± 12.34 ng/l, respectively, $P > 0.05$; and PGE2, 8.67 ± 2.15 ng/l versus 10.32 ± 2.65 ng/l, respectively, $P > 0.05$).

Serum concentrations of EGF and PGE2 in patients with LGIN were not significantly associated with disease endoscopic classification but there was a significant association with disease pathological severity (Table 3). Patients with severe LGIN had significantly higher concentrations of serum

| Study group          | H. pylori infection | n     | Yes          | No           |
|----------------------|---------------------|-------|--------------|--------------|
| Healthy control      | 548                 | 321 (58.58) | 227 (41.42) |
| Superficial gastritis| 3158                | 1495 (47.34) | 1663 (52.66) |
| Erosive gastritis    | 1752                | 475 (27.11)  | 1277 (72.89) |
| Hemorrhagic gastritis| 548                 | 321 (58.58) | 227 (41.42) |
| Atrophic gastritis   | 180                 | 41 (22.78)   | 139 (77.22)  |

Data presented as n (%) prevalence. Between-group comparisons of the H. pylori infection rate were performed using $\chi^2$-test.

| Chronic gastritis severity | Mild | Moderate | Severe |
|----------------------------|------|----------|--------|
| H. pylori infection status | $n = 2957$ | $n = 2416$ | $n = 265$ |
| Negative                  | 1559 (52.72) | 771 (31.91) | 2 (0.75) |
| Positive                  | 1398 (47.28) | 1645 (68.09) | 263 (99.25) |

Data presented as n (%) prevalence. Between-group comparisons of the H. pylori infection rate were performed using $\chi^2$-test.
EGF (P < 0.01), and lower concentrations of serum PGE2 (P < 0.01) than patients with mild or moderate disease (Table 3).

**Discussion**

The current study showed that prevalence of *H. pylori* infection was higher in patients with chronic gastritis than in healthy controls, in the Han Chinese population of central China. In patients with chronic gastritis, the prevalence of *H. pylori* infection was higher in patients with erosive gastritis compared with superficial gastritis, higher in patients with severe inflammation than in patients with mild or moderate inflammation, and higher in patients with LGIN compared with non-LGIN.

The current results suggested that the prevalence of *H. pylori* infection may be associated with the status of inflammation of chronic gastritis, and might present a risk effect for gastric precancerous lesions. A study of Romanian patients with chronic gastritis showed that all histological changes were strongly related to *H. pylori* infection and *H. pylori* infection was higher in patients with gastric intraepithelial neoplasia versus those without gastric intraepithelial neoplasia.

Intestinal metaplasia is associated with *H. pylori* infection, smoking, bile reflux and a high-salt diet. *H. pylori* infection might be implicated in the pathogenesis of gastric mucosal atrophy and intestinal metaplasia by induction of the host inflammatory and immune response, or by cytotoxins, such as vacuolating cytotoxin A or cytotoxin-associated gene A. *H. pylori* infection has been suggested to cause an imbalance between apoptosis and proliferation in normal gastric epithelial cells, which is reversible after *H. pylori* eradication. In addition, intraepithelial neoplasia has been shown as an important step in the occurrence of gastric cancer induced by *H. pylori* infection. In general, *H. pylori* infection is thought to cause gastric cancer in an indirect way.

The current study provided evidence that patients with *H. pylori*-positive LGIN had significantly higher serum EGF and lower serum PGE2 concentrations than patients with *H. pylori*-negative LGIN. Serum concentrations of EGF and PGE2 in patients with LGIN were analysed following stratification by endoscopic classification and disease severity. Serum EGF and PGE2 concentrations in patients with LGIN were not significantly associated with endoscopic disease classification, but there was a

**Table 3.** Serum concentrations of epidermal growth factor (EGF) and prostaglandin E2 (PGE2) in patients with low-grade intraepithelial neoplasia (LGIN; n = 82) with or without *H. pylori* infection, stratified by endoscopic classification and pathological severity.

| Endoscopic classification | H. pylori positive | H. pylori negative | H. pylori positive | H. pylori negative |
|---------------------------|---------------------|---------------------|---------------------|---------------------|
| EGF, ng/l                 |                     |                     |                     |                     |
| Superficial gastritis     | 16                  | 192.07 ± 50.88      | 70.41 ± 22.31       | 11.52 ± 1.44       |
| Erosive gastritis         | 52                  | 175.27 ± 45.85      | 66.82 ± 13.46       | 11.81 ± 1.96       |
| Haemorrhagic gastritis    | 9                   | 187.56 ± 66.81      | 75.87 ± 9.61        | 11.45 ± 1.95       |
| Atrophic gastritis        | 5                   | 191.91 ± 85.77      | 61.88 ± 22.55       | 13.41 ± 1.19       |
| Severity                  |                     |                     |                     |                     |
| Mild                      | 34                  | 134.93 ± 20.56      | 54.53 ± 11.40       | 12.94 ± 1.83       |
| Moderate                  | 42                  | 204.78 ± 31.97      | 77.17 ± 4.35        | 11.24 ± 1.25       |
| Severe                    | 6                   | 274.20 ± 6.37       | 86.5 ± 10.59        | 9.14 ± 0.35        |
| PGE2, ng/l                |                     |                     |                     |                     |

Data presented as mean ± SD. Continuous variables were statistically analysed using one-way analysis of variance.
significant association with disease severity. In patients with LGIN, significantly higher concentrations of serum EGF and lower concentrations of serum PGE2 were found with severe disease compared with mild or moderate disease; the authors speculate that abnormal levels of EGF and PGE2 might be involved in the pathogenesis of LGIN.

Epidermal growth factor is a polypeptide that stimulates or inhibits the proliferation of various cell types, and can stimulate the synthesis of mRNA, DNA and protein in epithelial cells to promote gastric mucosal epithelial hyperplasia.\textsuperscript{14} High levels of serum EGF and low levels of serum PGE2 have been observed in rats with chronic atrophic gastritis.\textsuperscript{15} Moreover, levels of serum EGF in rats with chronic atrophic gastritis were found to be associated with high levels of EGF receptor (R) expression.\textsuperscript{15,16} Abnormally high EGF expression has been observed in the transition region of paracarcinoma tissue, dysplastic cells and tumour cells,\textsuperscript{17} and EGF has been found to stimulate urokinase plasminogen activator receptor expression and cell invasiveness through extracellular signal regulated kinase, activator protein-1, and nuclear factor-\kappa\B signallng.\textsuperscript{18} \textit{H. pylori} infection has been indicated as a risk factor for LGIN,\textsuperscript{19,20} and \textit{H. pylori} may also stimulate the process of compensatory proliferation by promoting epithelial-cell apoptosis.\textsuperscript{21}

Prostaglandin E2 is generally considered to be a cell-protecting factor involved in the repair of damaged gastric mucosal cells by inhibiting secretion of gastric acid and increasing blood flow to the gastric mucosa.\textsuperscript{22} \textit{H. pylori} infection increases the number of inflammatory cells in gastric mucosa, results in the production of many inflammatory mediators and impairs cellular immune function of the gastric mucosa,\textsuperscript{23} which may have been the cause of decreased PGE2 levels observed in \textit{H. pylori}-positive patients with LGIN in the current study.

The current study is limited by small sample size and lack of functional investigation of EGF and prostaglandin E2, such as the mechanisms of interaction between signalling from prostaglandin receptors and EGFR in gastric mucosal cells. Therefore, further studies with a larger sample size are necessary to explore the biological mechanism and investigate the specific biomarkers of gastric precancerous lesions.

In conclusion, in this small-scale study, the prevalence of \textit{H. pylori} infection was higher in Han Chinese patients from central China who had chronic gastritis, compared with healthy controls, in the current study. In addition, \textit{H. pylori} infection was associated with chronic gastritis type (classified by endoscopy) and disease severity. In addition, high levels of serum EGF and low levels of serum PGE2 were observed in \textit{H. pylori}-positive patients with LGIN, and were associated with disease severity.

**Declaration of conflicting interest**

The authors declare that there are no conflicts of interest.

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