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Helicobacter pylori infection and gastric cardia cancer in Chaoshan region

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Abstract

Helicobacter pylori (*H. pylori*) infection represents the most important risk factor for gastric cancer, while its association with gastric cardia cancer (GCC) has not been recognized yet. In this current study, we aim to investigate the status of *H. pylori* infection in the gastric cardia tissue samples from high-risk populations in Chaoshan littoral region, and the relationship between *H. pylori* infection and chronic inflammation as well as the proliferative activity of the gastric cardia epithelial cells. A total of 706 gastric cardia biopsy specimens were obtained from 372 GCC cases and 334 tumor-free controls in Chaoshan littoral, a high-risk region for esophageal and gastric cardia cancer. Immunohistochemistry and Giemsa staining were employed for the verification of *H. pylori* infection. *H. pylori* infection rate was significantly higher in GCC (81.5%, $P < 0.01$) and gastric carditis (80.1%, $P < 0.01$) in comparison with that in the healthy group (34.8%). A significant higher prevalence of chronic inflammation was found in *H. pylori*+ samples (96.9%) than that in *H. pylori*− specimens (80.5%) ($P < 0.01$). To explore the possible role of *H. pylori* infection-related chronic inflammation in the GCC, we found that the expression of Ki-67 was progressively increased in tissues with chronic inflammation degrees from normal to severe inflammation ($P < 0.01$). Collectively, these results suggest that persistent *H. pylori* infection and the related chronic inflammation may contribute to the high incidence of GCC in Chaoshan littoral.

Keywords: Gastric cardia cancer; *Helicobacter pylori*; Inflammation; Ki-67

1. Introduction

Gastric cancer represents the second leading cause of cancer-related death worldwide, taking a toll of approximately 738,000 people in 2008 alone [1]. Gastric cancer was once considered as a single entity. Now, scientists divide this cancer into two main types: gastric cardia cancer and non-cardia gastric cancer (cancer in all other areas of the stomach). The incidence of GCC is characteristic of remarkable geographic aggregation, which resembled that of the esophageal squamous cell carcinoma (ESCC), particularly in high risk areas [2,3]. With widespread use of endoscopy and the improvement of lesion classification, more and more GCC were confirmed in ESCC high risk areas. The geographical similarity of esophageal cancer (EC) and GCC suggests that they share some common etiologies. Many environmental factors have been investigated in relation to EC [4]. Among them, diet has received particular attention as a critical contributing factor for the high EC incidence. Our previous study suggested that *Helicobacter pylori* infection may be an original cause leading to atypical hyperplasia of esophageal squamous epithelial

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tissues, and contributed to pathological carcinogenesis of ESCC [5].

H. pylori infection has been confirmed as an important factor for distal gastric cancer, however, its relationship with GCC still remains controversial [6–8]. Compared with gastric body and antrum, mucosal cellular structure and pH environment in gastric cardia are special, which may affect the infection and colonization of *H. pylori* and therefore the role of *H. pylori* in gastric cardia may differ from other parts of the stomach. For nearly two decades, the prevalence of *H. pylori* infection in developed countries is in decrease, and so is the incidence of distal gastric cancer. However, there was an increased incidence of GCC [9]. A report from Iran indicated that *H. pylori* infection is the main risk factor for gastritis for all sites of the stomach including gastric cardia [10]. However, it is still unclear whether *H. pylori* infection is really correlated with GCC in Chaoshan region, an area with high prevalence of gastric cardia cancer.

In this study, we investigate the status of *H. pylor*i infection in the gastric cardia tissue samples from high-risk populations in Chaoshan littoral, and the relationship between *H. pylor*i infection and chronic inflammation as well as the proliferative activity of the gastric cardia epithelial cells.

2. Materials and methods

2.1. Patients and sample collection

A total of 706 gastric cardia tissue samples were collected from 372 GCC patients and 334 tumor-free controls in Chaoshan (southern China) GCC high-risk region from January 2008 until December 2010. The 372 GCC patients included 58 females and 314 males. The 334 non-cancer controls were comprised of 110 women and 224 men without GCC. The median age was 62 for GGC patients and 56 for the controls. According to histological type, the 372 GCC samples were classified as follows: 321 tubular adenocarcinomas, 37 mucous adenocarcinomas, 11 adeno-squamous carcinomas, 2 papillate adenocarcinomas, and 1 undifferentiated carcinoma. In addition, the matched tumor-adjacent mucosa and distant tumor-free mucosa were collected in 119 individuals out of the 372 patients. Among the 334 control subjects, 23 were normal and 311 had chronic inflammation in the gastric cardia. All specimens were fixed in 10% neutral formalin solution, dehydrated and embedded in paraffin, and their pathological features and diagnosis were verified by two pathologists with hematoxylin and eosin staining.

H. pylori infection was diagnosed by immunohistochemistry and Giemsa staining. Infection was considered positive if both staining were positive (Fig. 1).

This study was approved by the Ethics Committee of Shantou University Medical College and informed consent was obtained from participating patients.

2.2. Giemsa staining

For the study, 4-μm sections were deparaffinized in xylene and rehydrated in a descending series of ethanol solutions, and then incubated with 0.5% hydrochloric acid alcohol for 10 min. After washing in distilled water, the slides were immersed in Giemsa stain preheated to 58 °C for 4 min, and immediately washed in distilled water. An additional wash in distilled water was required after treating with 1% glacial acetic acid for 3 s, and then the slides were placed into an incubator at 52 °C for 30 min. Sections were dehydrated, cleared in xylene, and mounted.

2.3. Immunohistochemistry

Paraffin-embedded samples were sectioned at 4 μm, mounted on gelatin-coated slides, dried at 60 °C for 3 h. The sections were deparaffinized in xylene, rehydrated in a

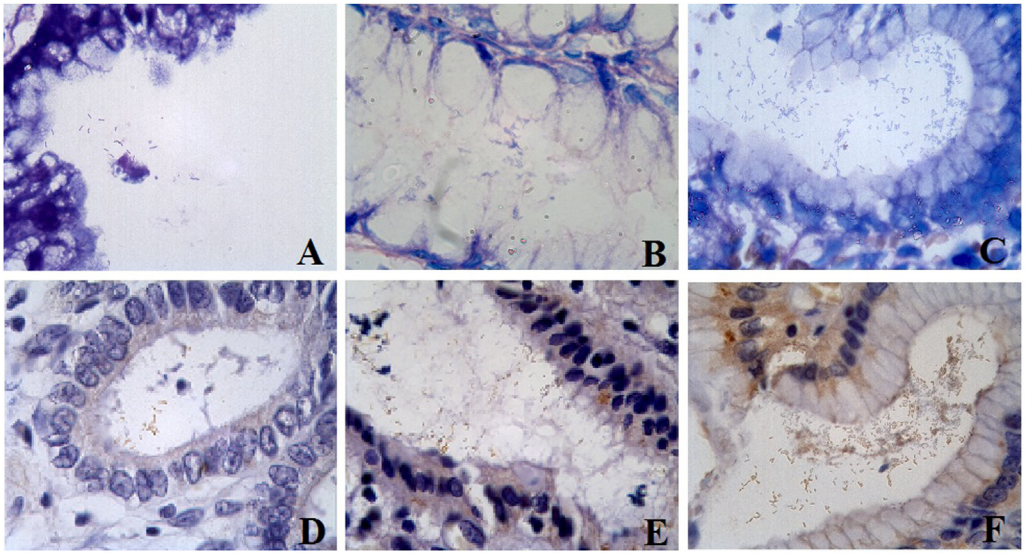

Fig. 1. *H. pylori* infection was identified by Giemsa and immunohistochemistry staining. Bacterial density was graded according to the Updated Sydney System. Representative images of *H. pylori* infection detected by Giemsa staining (A +; B ++; C +++) and IHC (D +; E ++; F +++), original magnification × 1000.