Helicobacter pylori and Telomerase Activity in Intestinal Metaplasia of the Stomach

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Background: Helicobacter pylori (H. pylori) has been considered a definitive carcinogen in gastric cancer. Telomerase is activated in gastric cancer and some premalignant gastric lesions, including intestinal metaplasia (IM). In this study, we evaluated the relationships of both H. pylori infection and telomerase activity with endoscopic and histologic features in IM. The effects of H. pylori eradication on endoscopic, histologic and biochemical changes were evaluated.

Methods: Endoscopic biopsies were obtained from 43 patients with IM for rapid urease, histologic and telomerase tests. The endoscopic and histologic features, H. pylori infection and telomerase were assessed. After H. pylori eradication, 15 patients were re-evaluated and compared after 4 months.

Results: Thirty-four (79.1%) patients were infected with H. pylori. The incidence of H. pylori infection was borderline correlated to the severity of IM (p=0.078). Telomerase was elevated in eight (18.6%) patients. Telomerase tends to be high in subtype III and endoscopic grade III of IM. After H. pylori eradication, endoscopic extent (p=0.039) and histologic severity (p=0.074) showed improvements, and telomerase decreased significantly (p=0.0001).

Conclusion: Our data suggest that telomerase is associated with the severity and extent of IM and that H. pylori eradication improves the endoscopic and histologic features in IM, and decreases telomerase activity. H. pylori eradication can be considered one of the methods to prevent gastric cancer in patients with H. pylori-infected IM. Further long-term and large-scaled study will be needed.

Key Words: Telomerase. Helicobacter pylori. Intestinal metaplasia. Gastric cancer

INTRODUCTION

Gastric cancer is one of the most common types of cancer worldwide1). Despite some improvement in its treatment, the 5-year survival rate of gastric cancer is still low. The prevalence of intestinal metaplasia (IM) is closely related to H. pylori infection2). Furthermore, chronic gastritis due to H. pylori infection may progress to IM and even gastric cancer3). H. pylori-induced inflammation facilitates gastric carcinogenesis by increasing the rate of cell replication, decreasing the concentration of ascorbic acid and inducing DNA damage via reactive oxygen species4, 7). Both environmental and genetic factors are crucial in the multistage model of gastric tumorigenesis4). A preliminary observation has suggested that both IM and H. pylori infection are important targets in the prevention of gastric cancer5).

Telomerase, located at the distal end of human chromosomes, comprises simple, repetitive and G-rich
hexameric sequences (TTAGGG), and is vital for chromosomal stability and replication. Telomerase is a ribonucleoprotein polymerase that adds telomeric sequences onto the ends of the chromosome to compensate for the DNA end-replication problem. Thus, this activity is strongly associated with cell immortalization and carcinogenesis, and its presence is elevated in 85% of human cancers, including those associated with the stomach, colon, breast, bladder, prostate, liver, lung and ovary. The measurement of telomerase activity may become a diagnostic tool for cancer\(^5\). However, telomerase activity can also be found in precancerous lesions, such as IM\(^6\).

In this study, we evaluated the relationships of both \(H.\) pylori infection and telomerase activity with endoscopic and histologic features in IM. The effects of \(H.\) pylori eradication on endoscopic, histologic and biochemical changes were evaluated.

**PATIENTS AND METHODS**

This study involved 43 patients who, between January 1999 and June 2000, were diagnosed with IM without any gastroenterologic diseases by an upper gastrointestinal endoscopic examination at a gastrointestinal endoscopy unit of Soochunhyang University Chonan hospital. Thirty-four of these patients were diagnosed as having \(H.\) pylori infection. Eight patients exhibited elevated telomerase activity. Twenty-three of the patients with \(H.\) pylori infection were treated with an \(H.\) pylori eradication regimen and fifteen of them were able to be followed up for changes in endoscopic, pathologic and telomerase results after 4 months of complete therapy.

1) **Gastric tissue sampling by upper gastrointestinal endoscopy**

Six gastric specimens were obtained by endoscopic biopsy of the IM lesion. Two of these specimens were fixed in 10% buffered formalin, embedded in paraffin and sectioned, and then one was stained with hematoxylin–eosin (H & E) and/or Warthin-Starry silver stain for detecting \(H.\) pylori. The other specimens were stained using the high–iron diamine (HID)/alcian blue (AB) technique to differentiate acidic mucin into sialomucins (blue) and sulfomucins (brown–black)\(^7\). The other two specimens were used to detect telomerase activity. One set of gastric tissue from the antrum and body was obtained and used for the Campylobacter Like Organism (CLO) test and monitored for color changes for up to 24 h at room temperature.

2) **Grading of intestinal metaplasia**

Endoscopic grades were classified according to the degree and pattern of mucosal elevation: I, granular; II, nodular; III, irregular or confluent. The extent of IM, as assessed endoscopically, was graded as follows: I, confined to antrum; II, extending to lower body; III, extending to upper body and fundus. Histologic grading is classified by HID/AB and H&E staining. Metaplastic lesions were classified into three subtypes: type I, complete IM characterized as resembling the normal intestinal epithelium; type II, incomplete IM expressing sialomucins but not sulfomucin; and type III, incomplete IM expressing sulfomucins. If IM of more than one subtype was present in a given sample, the case was assigned to the least mature subtype, as proposed previously\(^2\).

3) **Telomerase assay**

Protein extracts were obtained and a telomerase activity assay was performed as described previously\(^8\). One to three milliliters of tissue extract (1–50 \(\mu\)g of total protein) was subjected to a modified, semiquantitative telomeric repeat amplification protocol, which included an internal telomerase assay standard\(^9\). A polymerase chain reaction (PCR) was performed using a thermal cycler (DNA engine: Peltier thermal cycler, MJ Research, Waltham, Mass., USA) using primer elongation at 25\(^\circ\)C for 10 min and inactivation at 94\(^\circ\)C for 5 min. Thirty–three PCR cycles were performed which consisted of 94\(^\circ\)C for 30 s, 50\(^\circ\)C for 30 s and 72\(^\circ\)C for 90 s. After hybridization using digoxin and a telomeric repeat sequence, the absorbancy was measured at 450 nm by a microtiter plate (ELISA) reader. Negative and positive controls were read using a PCR ELISA kit (TelomAGG Telomerase PCR ELISA\(^{flus}\), Roche, Nutley, N.J., USA). The final value was obtained by subtraction of the mean of the absorbance readings of the negative controls from the absorbance readings of the samples (i.e., AsAs.o). Samples are considered as telomerase–positive if the difference in absorbance (A) is higher than two times the background activity. Quantification of telomerase activity was determined as the relative telomerase activity using a formula provided in the PCR ELISA kit.

4) **\(H.\) pylori eradication and follow-up**

The \(H.\) pylori eradication regimen was administrated to