Decreased Gastric Proliferation of Foveolar Epithelial Cells after the Eradication of *Helicobacter pylori*

Increased epithelial cell proliferation is associated with an increased risk of gastric carcinoma. *Helicobacter pylori* infection is an established risk factor for gastric cancer and the organism has recently been classified as a group I carcinogen by an IARC working group. In this study, we describe differences in gastric epithelial cell proliferation between a *H. pylori* eradicated group (n=21) and a not eradiacted group (n=8) after anti-*H. pylori* eradication therapy to show that increased cell proliferation is associated with *H. pylori* infection. *H. pylori* infection was determined by rapid urease test and immunohistochemical method with anti-*H. pylori* polyclonal antibody. Gastric epithelial cell proliferation was assessed using immunohistochemical method using Ki-67 monoclonal antibody. Ki-67 positive cells in *H. pylori* associated chronic active gastritis were observed in the glandular neck and the upper portion of foveolar epithelium. Patients who cleared their *H. pylori* infections showed a significant decrease of Ki-67 labeling index after therapy (0.73±0.10 vs. 0.48±0.08, $p<0.01$). By contrast, Ki-67 labeling index before and after treatment in patients who remained positive for *H. pylori* showed no significant difference (0.78±0.08 vs 0.74±0.10, $p>0.05$). These results indicate that *H. pylori* infection increases the proliferation of gastric foveolar epithelium, which is reduced by the eradication therapy. We suggest that anti-*H. pylori* eradication therapy can prevent mucosal cell proliferation to be closely associated with gastric carcinogenesis. ***(JKMS 1997; 12: 421~6)***

Key Words : *Helicobacter pylori; Gastrointestinal neoplasms; Risk factors; Eradication therapy*

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INTRODUCTION

Helicobacter pylori infection is an established risk factor for gastric cancer (1~3) and the organism has recently been classified as a group I carcinogen by an IARC working group (4). *H. pylori* infection is the main cause of chronic gastritis which results in atrophy and intestinal metaplasia of the underlying mucosa in many patients (5). Both these conditions are known to be associated with an increased risk of gastric carcinoma (6).

Many irritants are thought to increase cancer risk both through induction of inflammation and through increased proliferation. Inflammatory cells can induce genotoxic effects including DNA strand breaks (7, 8), sister chromatid exchange (8), mutation (9, 10), and neoplastic transformation (11, 12). The effects of increased proliferation resulting from the repair of the damaged mucosa also contribute to genetic damage. Rapidly dividing cells are known to be at increased risk for undergoing mutation when compared to quiescent cells (13). Furthermore, cell proliferation is required to fix genetic damage within the cell population (14).

One of the most widely studied proliferation-associated markers is the Ki-67 monoclonal antibody that recognizes a labile epitope on a nuclear antigen in cycling cells, and the antigen is expressed in all active parts of the cell cycle (15). The drawback of this antibody is that it works only on frozen section. Recently, the characterization of the protein recognized by the Ki-67 antibody has enabled production of new monoclonal antibodies with recombinant technology (16, 17). One of these antibodies, MIB1, can be used on formalin-fixed, paraffin-embedded tissue, thus overcoming the main problem of Ki-67 monoclonal antibody.

In this study, we describe differences in gastric epithelial cell proliferation between a *H. pylori* eradicated group and a not eradicated group after anti-*H. pylori* eradication therapy to show that increased cell proliferation is associated with *H. pylori* infection.

MATERIALS AND METHODS

Patient selection

This study was based on the analysis of 29 patients referred for upper gastrointestinal endoscopy at the DongGuk University Kyungju Hospital. Sixteen patients were men and 13 were women (age range 14~90 years; mean±SD=39.8±16.1 years). All these patients had been infected with *H. pylori,* which was determined by rapid urease test (Tri-Med Specialties Inc, U.S.) and immunohistochemical method using anti-*H. pylori* polyclonal antibody. Amoxicilline (500 mg 3 times daily for 7 days), metronidazole (500 mg 3 times daily for 7 days), together with omeprazole (20 mg 2 times daily for 7 days), and thereafter omeprazole (20 mg 1 time daily for 14 days) were prescribed for all patients.

Endoscopic biopsy

Three or four antral biopsies were performed before and after therapy. The period between first and follow-up biopsy was 27~160 days. One antral biopsy specimen was used for rapid urease test for *H. pylori.* The urease test was considered as positive when the urea solution changed color from yellow to pink at room temperature within 24 h. The remaining two or three biopsy specimens were used for routine histology.

Histopathological analysis

Two or three pieces of the biopsy specimen were fixed in 10% buffered formalin, embedded in paraffin. Five-micron thick sections were cut from each paraffin block. Sections were intially stained with H&E for routine histology. Neutrophil infiltration of gastric mucosa was graded as follows : 0, no neutrophils; 1, <10 neutrophils/HPF; 2, >10 neutrophils/HPF, but infiltration involving <50% of mucosal surface; and 3, neutrophil infiltration involoving >50% of the mucosal surface. Lymphocytic infiltration was graded as follows: 1, mild and focal; 2, dense but focal; and 3, dense and diffuse lymphocytic infiltration.

Immunohistochemical analysis

Two five-micron thick sections of each specimen were mounted on a positively charged slide, deparaffinized in xylene, and rehydrated through decreasing concentraton of ethanol. The slides were immersed in a pressure cooker with 0.01 M citrate buffer (pH 6.0) in the autoclave for 3-5 minutes, and then blocked with normal horse serum and incubated for 1 hour with anti-*H. pylori* polyclonal antibody (1:200 in TBS : BA71, Dako, Denmark) and the MIB1 monoclonal antibody specific for Ki-67 antigen (1:100 in TBS : Immunotech, Marseille, France). Bound antibody was detected by incubation of section for 30 minutes with a biotinylated horse antimouse antibody (1:200 in TBS : Dako, Denmark) followed by streptavidin-horseradish peroxidase complex (1:100 in TBS: Dako, Denmark). Prior to the addition of each antibody, the slides were washed extensively in TBS. Color was developed with 3-amino-9-ethylcarbazole, and sections were counterstained with hematoxylin before mounting. The density of *H. pylori* was divided into four grades (grades 0, I, II, III) by the updated sydney system (18). The calculation of Ki-67 antigen labeling index (Ki-67 LI) was done by counting cells in well labeled areas of the glandular necks in nonmetaplastic areas, as determined by scanning at low magnification. Actual counts were made at x400 magnification. Nuclei with any detectable staining above background levels were scored as positive. A total of 500 nuclei were evaluated, and positive nuclei were expressed as a percentage. The correlation between *H. pylori* grade and Ki-67 LI was assessed.

Statistical analysis

Pretreatment and posttreatment differences in Ki-67 LI, and inflammatory reaction were analyzed using the paired t-test, Wilcoxon signed rank sum test, χ^2 test, Fisher's exact test, and regression linear analysis. Statistical significance of difference was determined by $p<0.05$.

RESULTS

Among the eight patients who did not clear their *H. pylori* infections, moderate and severe infiltration of neutrophil and mononuclear cell before and after treatment were eight, and there was no significant change of infilration of neutrophil and mononuclear cell ($p>0.05$, Table 1). Among the 21 patients who cleared their *H. pylori* infections, moderate and severe infiltration of neutrophil and mononuclear cell before treatment was 21 and 20, respectively, and mild and absent infiltration of neutrophil and mononuclear cell after treatment was 20 and 14, respectively. Therefore, there was a significant decrease of infiltration of neutrophil and mononuclear cells ($p<0.05$, Table 1).

Ki-67 antigen positive cells of *H. pylori* associated chronic active gastritis were observed in the glandular neck and the upper portion of foveolar epithelium (Fig. 1B). But Ki-67 antigen positive cells in patients who cleared their *H. pylori* infections were mainly observed

Table 1. Inflammatory reaction, before and after treatment, by *H. pylori* outcome

	Before Tx	After Tx
H. pylori not eradicated group*		
Neutrophil		
Absent & mild	0	0
Moderate & severe	8	8
Mononuclear cell		
Mild	0	0
Moderate & severe	8	8
H. pylori eradicated group▲		
Neutrophil		
Absent & mild	0	20
Moderate & severe	21	1
Mononuclear cell		
Mild	1	14
Moderate & severe	20	7

Tx : Treatment
H. pylori : *Helicobacter pylori*, * $p>0.05$, ▲ $p<0.01$

Table 2. Ki-67 labeling indices in gastric glandular neck, before and after treatment, by *H. pylori* outcome

	No	Before Tx	After Tx
H. pylori not eradicated group	8	0.78±0.08	0.74±0.10
H. pylori eradicated group*	21	0.73±0.10	0.48±0.08

Tx : Treatment
H. pylori : *Helicobacter pylori*
* $p<0.01$

Table 3. Ki-67 labeling indices in gastric glandular neck of follow-up biopsy according to period

Period(day)	*H. pylori* not eradicated group(No)	*H. pylori* eradicated group(No)
25< ≤35	0.72±0.10(5)	0.50±0.08(13)
35<	0.77±0.09(3)	0.47±0.08(13)

H. pylori : *Helicobacter pylori*
$p>0.05$

in the glandular neck portion (Fig. 2B). Patients who cleared their *H. pylori* infections (n=21) showed a significant decrease of Ki-67 LI after therapy (0.73±0.10 vs. 0.48±0.08, $p<0.01$) (Table 2). By contrast, Ki-67 LI before and after treatment in patients who remained positive for *H. pylori* infection (n=8) showed no significant difference (0.78±0.08 vs 0.74±0.10, $p>0.05$) (Table 2). The Ki-67 LI after therapy for the group that remained positive for *H. pylori* infection was significantly higher than that for the group that cleared *H. pylori* infection (0.74±0.10 vs. 0.48±0.08, $p<0.01$) (Table 2). The correlation between *H. pylori* grade and Ki-67 LI was assessed and it was statistically significant (Rsq= 0.6280, $p<0.001$) (Fig. 3).

Patients who did not clear their *H. pylori* infections showed no significant change of Ki-67 LI according to follow-up biopsy period (0.72±0.10 vs. 0.77±0.09, $p>0.05$) and also no significant change of Ki-67 LI was present in patients that cleared *H. pylori* (0.50±0.08 vs 0.47±0.08, $p>0.05$) (Table 3).

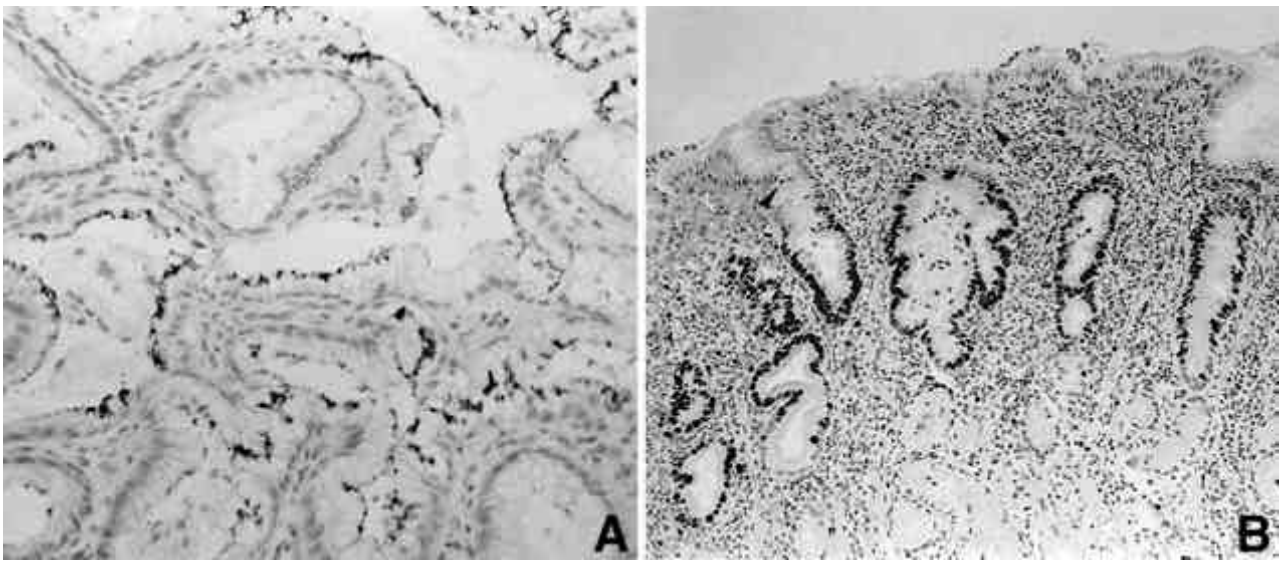

Fig. 1. Immunohistochemical staining for *H. pylori* (A) and Ki-67 antigen (B) before the eradication therapy. *H. pylori* associated chronic active gastritis shows increased Ki-67 antigen expression which is located in the upper foveolar segment (arrowhead) as well as the lower segment.

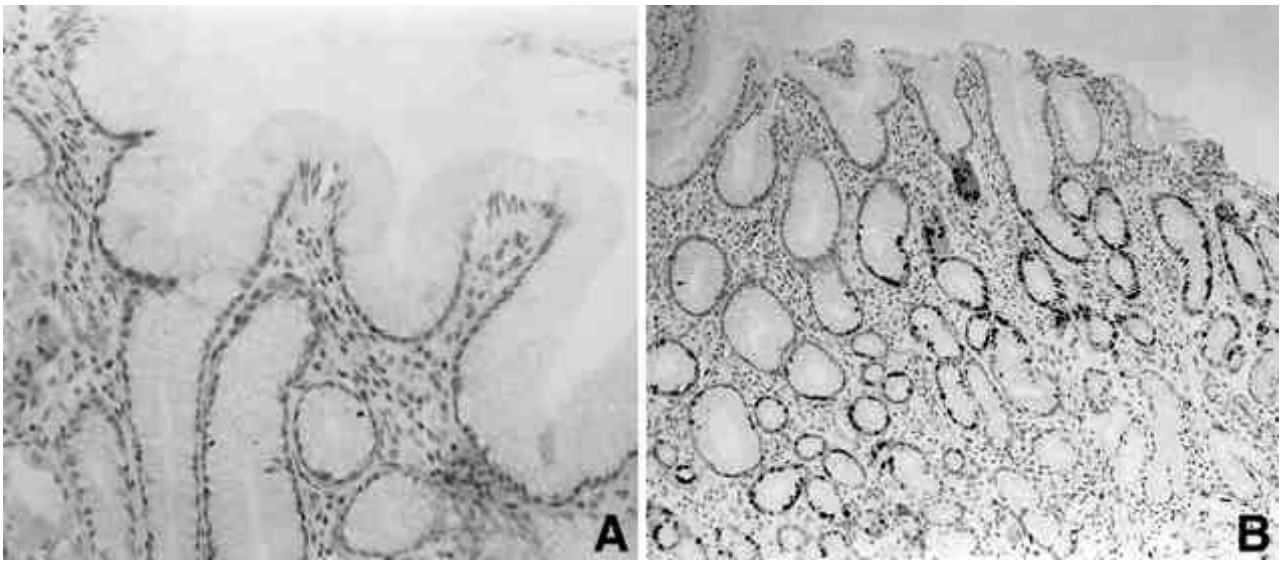

Fig. 2. Immunohistochemical staining for *H. pylori* (A) and Ki-67 antigen (B) after the eradication therapy. *H. pylori* is absent and decreased Ki-67 antigen expression is located in the lower foveolar segment.

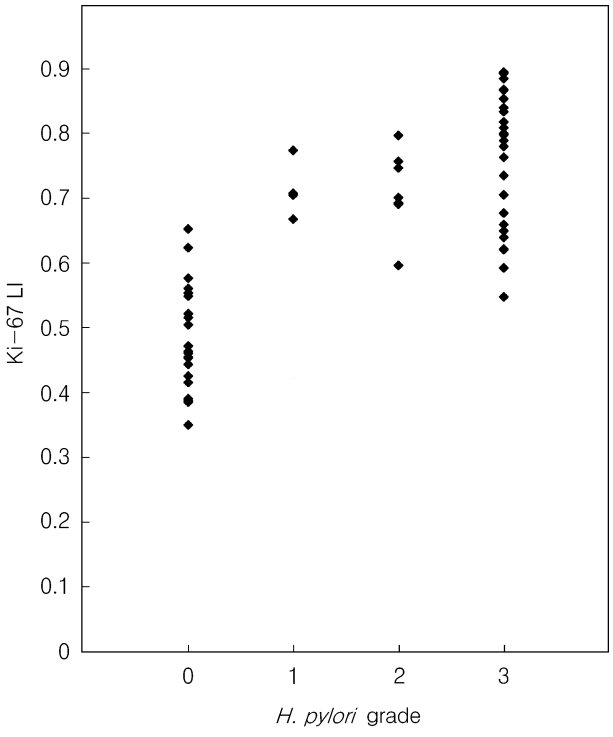

Fig. 3. The correlation between *H. pylori* grade and Ki-67 LI is significant (Rsq=0.6280, p<0.001).

DISCUSSION

In 1988 Correa and colleagues (19) postulated that gastric cancer develops through a complex sequence of events from normal mucosa to superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, and finally to intestinal type gastric carcinoma. Long term studies of *H. pylori* infection have provided evidence of a progression from *H. pylori* associated gastritis to atrophic gastritis, intestinal metaplasia, and dysplasia (5, 20). Epidemiological studies have suggested that intestinal type carcinoma is more influenced by environmental factors than diffuse type, which is thought in part to be familial or genetic (21). The role of *H. pylori* in these types of gastric carcinoma is controversial, however, recent studies have identified *H. pylori* as a risk factor for both types of gastric carcinoma (1, 2, 22).

This study demonstrates that the eradication of *H. pylori* infection is associated with a significant decrease of Ki-67 LI in the foveolar epithelium at the isthmic portion and inflammatory response of lamina propria, and correlation exists between *H. pylori* grade and Ki-67 LI. PCNA LI or AgNOR counts significantly decreased after successful eradication which reduced mucosal inflammation (23, 24). What are the factors which proliferate the gastric foveolar epithelium in the presence of *H. pylori* infection? Previous studies have shown that some products of *H. pylori* (25) and oxygen free radicals (26) produced by neutrophils and monocyte observed in *H. pylori* infection, damage gastric mucosal epithelium. Increased proliferation seems to result from the repair of damaged mucosa. Our study shows that Ki-67 antigen positive cells in *H. pylori* associated chronic active gastritis appear in the upper portion of the gastric foveolar epithelium, which usually does not contain cells outside the G_0 phase of the cell cycle (27). This finding suggests that in response to *H. pylori* infection, mucus producing

foveolar columnar epithelium is replaced by cells outside the phase G_0 phase of the cell cycle. Recently, using PCNA LI, Panella and colleagues (28) documented a progressive increase of epithelial proliferation in the successive stages of *H. pylori* infection ranging from gastritis alone to the development of incomplete intestinal metaplasia, a well-known precancerous condition, and expansion of proliferative compartments towards the upper foveolar third, which is compatible with our findings. In the epithelium of regenerative mucosa of patients with ulcerative colitis, Ki-67 immunoreactivity remained restricted to the base of crypts, but the replicative zone is expanded when compared with that of normal colon (29). It is known that proliferating cells are particularly sensitive to mutagenic factors (13, 30) and recent evidence has demonstrated that bacterial animal gastritis (*H. mustelae* infection in ferrets) is accompanied by an enhanced carcinogenic activity of N-methyl-N-nitro-N-nitrosoguanidine (31). In humans some substances that are introduced with food such as nitrosoamine (32), preservative (33), and additives (34) are particularly important as mutagens for the stomach. Alterations of the cell kinetics have been postulated as markers of gastric cancer risk (18, 35), and excessive cell replication is a prominent factor in carcinogenesis. Our results strongly suggest that excessive cell replication plays a role in the increased gastric cancer risk associated with chronic *H. pylori* infection. Recent study of gastric epithelial cell kinetics in the progression from normal mucosa to gastric carcinoma has suggested that *H. pylori* might be an initiating step in gastric carcinogenesis (36).

In conclusion, these results indicate that *H. pylori* infection increases the proliferation of gastric foveolar epithelium, which is reduced by eradication therapy. We suggest that anti-*H. pylori* eradication therapy can prevent mucosal cell proliferation to be closely associated with gastric carcinogenesis.

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