

## Expression of mutant type-*p53* products in *H pylori*-associated chronic gastritis

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Supported by Grants-in-Aid (C-14) from the Ministry of Health, Labor and Welfare, Japan

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Received: 2006-12-18 Accepted: 2007-02-25

### Abstract

**AIM:** To investigate the mutation of *p53* immunohistochemically in non-tumorous gastric mucosa with *H pylori* infection before and after *H pylori* eradication therapy.

**METHODS:** 53 subjects (36 male, 17 female, mean age  $\pm$  SEM,  $57.1 \pm 12.1$ ) undergoing endoscopic examination were included in this study. 42 of 53 patients were *H pylori*-positive, and 11 were *H pylori*-negative. All *H pylori*-positive patients had successful eradication therapy. Biopsy specimens were taken from five points of the stomach, as recommended by the updated Sydney system. Immunohistochemical studies were performed by using primary antibodies against *p53* (DO-7 and PAb240).

**RESULTS:** *p53* (DO-7 and PAb240) immunoreactivity was shown in the neck region of the gastric pits, however, quite a few cells were found to be immunopositive for *p53* (PAb240) in the *H pylori*-infected gastric mucosa. The proportion of patients immunopositive for *p53* (PAb240) was significantly reduced 6 mo after eradication [28/42 (66.7%) to 6/42 (14.3%)] ( $P < 0.05$ ), while the biopsies taken from *H pylori*-negative patients showed no immunoreactivity for *p53* (PAb240). *p53* (PAb240)-positive patients were divided into two groups by the number of positive cells detected: one with more than six positive cells per 10 gastric pits (group A,  $n = 12$ ), and the other with less than five positive cells per 10 gastric pits (group B,  $n = 30$ ). Atrophy scores in group A were significant higher than those in group B at the greater curvature of the antrum (group A:  $2.00 \pm 0.14$  vs group B:  $1.40 \pm 0.15$ ,  $P = 0.012$ ), the lesser curvature of the corpus (group A:  $2.00 \pm 0.21$  vs group B:  $1.07 \pm 0.23$ ,  $P = 0.017$ ), and the greater curvature of the corpus (group A:  $1.20 \pm 0.30$

vs group B:  $0.47 \pm 0.21$ ,  $P = 0.031$ ). Group A showed significant higher intestinal metaplasia scores than group B only at the lesser curvature of the antrum (group A:  $2.10 \pm 0.41$  vs group B:  $1.12 \pm 0.29$ ,  $P = 0.035$ ).

**CONCLUSION:** *H pylori*-associated chronic gastritis expressed the mutant-type *p53*, which was significantly associated with more severe atrophic and metaplastic changes. *H pylori* eradication led to a significant reduction in the expression of the mutant-type *p53*. It is considered that *H pylori*-infected chronic gastritis is associated with a genetic instability that leads to gastric carcinogenesis, and *H pylori* eradication may prevent gastric cancer.

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**Key words:** *H pylori*; *H pylori* eradication; Atrophic gastritis; Mutant-type *p53*; Gastric cancer; Updated Sydney system

Kodama M, Murakami K, Okimoto T, Sato R, Watanabe K, Fujioka T. Expression of mutant type-*p53* products in *H pylori*-associated chronic gastritis. *World J Gastroenterol* 2007; 13(10): 1541-1546

<http://www.wjgnet.com/1007-9327/13/1541.asp>

### INTRODUCTION

*Helicobacter pylori* is one of the main pathogens that cause many kinds of gastroduodenal diseases. These include acute gastritis<sup>[1-3]</sup>, chronic atrophic gastritis<sup>[3,4]</sup>, intestinal metaplasia<sup>[5]</sup>, peptic ulcer<sup>[6,7]</sup>, mucosal associated lymphoid tissue (MALT) lymphoma<sup>[8]</sup>, and other disorders<sup>[9,10]</sup>. Although many epidemiological studies and animal models revealed close association between gastric cancer and *H pylori* infection<sup>[11-13]</sup>, there have been few studies that report on genetic alterations suggestive of gastric carcinogenesis associated with chronic *H pylori* infection<sup>[14-17]</sup>. Previously, we reported the expression of *p53*, a product of oncosuppressor-gene and cell cycle regulator, in the *H pylori*-infected human gastric mucosa<sup>[18]</sup> as well as in a Japanese monkey model<sup>[19]</sup>. *p53* expression was observed in the neck region of the gastric pit, and was reported to be reduced after *H pylori* eradication<sup>[18]</sup>. *p53* mutation is among the major episodes in the multi-step process of gastric carcinogenesis, while

it has also been reported in pre-malignant lesions of the stomach, such as chronic gastritis, intestinal metaplasia, and dysplasia<sup>[20-22]</sup>. However, it remains unclear whether *p53* expression in the gastric pit is mutant-type or wild-type. In this study, we used the primary antibodies reacting with both wild and mutant type-*p53*, and those reacting only with the mutant-type *p53* in our immunohistochemical studies to clarify the *p53* alterations in the *H pylori*-infected gastric mucosa.

## MATERIALS AND METHODS

### Subjects

A total of 53 subjects (36 male, 17 female, mean age SEM,  $57.1 \pm 12.1$ ) who underwent endoscopic examination were included in this study. 42 out of 53 patients were *H pylori*-positive and 11 were *H pylori*-negative. In 42 *H pylori*-positive patients, 14 patients had gastric ulcers, 5 had duodenal ulcers, 5 had gastro-duodenal ulcers, and 15 had chronic gastritis. All *H pylori*-positive patients had successful eradication therapy, and underwent biopsies before and six months after eradication. Biopsies for examination were taken at the same biopsy sites in *H pylori*-negative subjects as in the *H pylori* positive patients.

### Detection of *H pylori* in gastric biopsy specimens

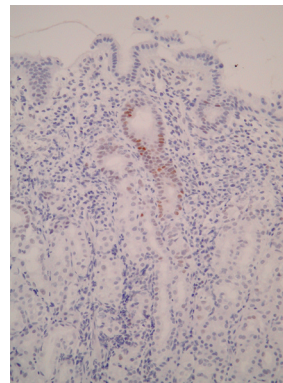
*H pylori* in the stomach was detected by the rapid urease test, culture, and histological examination. For the urease test, biopsy specimens were immediately inserted into the rapid urease test solution. For culture detection, biopsy material was cultured on 7% sheep's blood agar plates under micro-aerobic conditions and at high humidity and at 37°C for four days. *H pylori* was histologically detected by May-Giemsa stain. *H pylori* eradication was considered successful when the results of all three tests were found negative.

### Histological evaluation

Biopsy specimens were taken from five points of the stomach, as recommended by the updated Sydney system<sup>[23]</sup>, i.e. the lesser curvature of the antrum (A1), and the greater curvature of the antrum (A2), the smaller curvature of the angle (IA), and the lesser curvature of the middle corpus (B1), and the greater curvature of the upper corpus (B2). All biopsy materials were fixed in buffered formalin for 24 h and embedded in paraffin. Serial sections were stained with haematoxylin-eosin and with May-Giemsa stain. The status of the gastric mucosa was evaluated according to the updated Sydney system. The degree of inflammation, neutrophil activity, atrophy, and intestinal metaplasia were classed by four grades, with 0 being for 'normal', 1 for 'mild', 2 for 'moderate', and 3 for 'marked', respectively.

### Immunohistochemical detection of *p53*

Serial paraffin sections were washed in 1/15 mol/L phosphate buffered saline (PBS, pH 7.4) three times for five minutes, and pre-incubated in normal rabbit serum (1:10 in PBS) for 20 min. Next, these sections were incubated with primary antibodies for 16 h at 4°C, followed



**Figure 1** Immunohistochemistry for *p53* (DO-7) in *H pylori*-infected gastric mucosa. Positive staining for *p53* (DO-7) was observed in the gastric pit, especially in the neck region before eradication ( $\times 200$ ).

by the avidin-biotin complex method. The sections were immersed in 0.05 mol/L Tris-HCl buffer containing 0.02% 3, 3'-diaminobenzidine tetrahydrochloride and 0.005%  $H_2O_2$ , and the nuclei were counterstained with hematoxylin. Control sections incubated with normal mouse IgG instead of the primary antibody showed no non-specific staining. The primary antibodies used in this study were mouse monoclonal anti-*p53* protein (clone DO-7 and PAb240, Dako, Carpinteria, CA, USA), and were diluted to 1:50. Control sections incubated with normal mouse IgG instead of the primary antibody showed no non-specific staining.

### Evaluation of *p53* (DO-7 and PAb240)

Cells immunopositive for *p53* (DO-7) were counted in longitudinal sectioned foveolar pits and were found visible along their whole length. Labeling indices for *p53* (DO-7) are indicated as the proportion of positive cells among the cell total of the gastric mucosa. At least 500 cells in the gastric epithelial cells were calculated in each specimen. Cells immunopositive for *p53* (PAb240) were counted in per 10 longitudinal gastric pits.

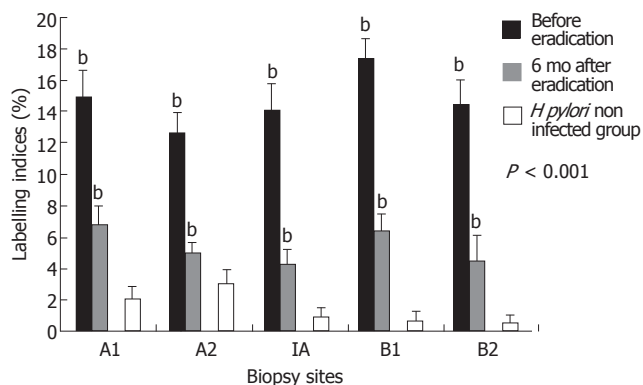
### Statistical analysis

Statistical analysis (Wilcoxon signed-rank test and Mann-Whitney's U test) was performed to analyze the updated Sydney system score and labeling indices.

## RESULTS

### Immunohistochemical detection of *p53* (DO-7)

Immunoexpression of *p53* (DO-7) was observed in the neck region of the *H pylori*-infected gastric pits from all biopsy sites (Figure 1). These *p53*-positive cells were significantly reduced 6 mo after *H pylori* eradication. In contrast, the gastric mucosa without *H pylori* infection showed very few positive cells in the gastric pits. The labeling index for *p53* (DO-7) in the *H pylori*-infected group before eradication was significantly higher than that in the non-*H pylori*-infected group ( $P < 0.001$ ) (Figure 2). After eradication, the labeling index for *p53* was significantly reduced at all biopsy sites (A2; from 14.98% to 6.80%;  $P < 0.001$ , A1; from 12.63% to 4.96%;  $P < 0.001$ , IA; from 14.24% to 4.26%;  $P < 0.001$ , B1; from 17.49% to 6.41%;  $P < 0.001$ , B2; from 14.45% to 4.48%;  $P < 0.001$ ) (Figure 2).

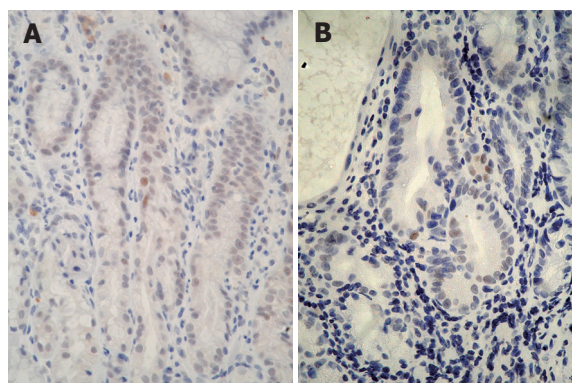


**Figure 2** Labeling indices for *p53* (DO-7) in 42 patients with *H pylori* infection, 6 mo after eradication, and 11 patients without *H pylori* infection. Results were shown as mean  $\pm$  SEM. *p53* (DO-7) indices were significantly reduced 6 mo after eradication at all biopsy sites. *p53* indices for the *H pylori*-infected mucosa were significantly higher than those for the gastric mucosa without *H pylori* infection at all biopsy sites. <sup>b</sup> $P < 0.001$ , vs *H pylori* non-infected group.

### Immunohistochemical detection of *p53* (PAb240)

Immunoreactivity of *p53* (PAb240) was also shown in the neck region of the gastric pits; however, quite a few cells were found to be immunopositive for *p53* (PAb240) in the *H pylori*-infected gastric mucosa (Figure 3A and 3B). Indices for *p53* (PAb240) in all examined patients are indicated in Table 1. Immunopositive cells for *p53* (PAb240) were found in 28 out of 42 (66.7%) *H pylori*-positive patients, while *H pylori*-negative patients showed no immunoreactivity for *p53* (PAb240) in all five biopsy specimens. Six months after eradication, the ratio of cells immunopositive for *p53* (PAb240) was significantly reduced [28/42 (66.7%) to 6/42 (14.3%)] ( $P < 0.05$ ) (Table 2). In patients immunoreactive for *p53* (PAb240), the total number of positive cells from all five biopsy specimens ranged from 0 to 19.1 per 10 gastric pits. When divided by the number of positive cells, 30 *H pylori*-positive patients had less than five cells, 8 patients had six to 10 cells, 1 patient had 11 to 15 cells, and 3 patients had more than 16 positive cells, respectively, with the majority of patients found to have less than five positive cells. Thus the patients were divided into two groups by the number of positive cells: one with more than six positive cells per 10 gastric pits (group A,  $n = 12$ ); and the other with less than five positive cells per 10 gastric pits (group B,  $n = 30$ ).

Before eradication, labeling indices of *p53* (DO-7) were significantly higher in group A than in group B at all biopsy sites except the lesser curvature of the antrum (Figure 4). *H pylori* density, inflammation, and activity scores in the updated Sydney system showed no significant difference between the groups (Table 3). However, atrophy scores in group A were significantly higher than in group B at biopsy site A2 (group A:  $2.00 \pm 0.14$  vs group B:  $1.40 \pm 0.15$ ,  $P = 0.012$ ), B1 (group A:  $2.00 \pm 0.21$  vs group B:  $1.07 \pm 0.23$ ,  $P = 0.017$ ), and B2 (group A:  $1.20 \pm 0.30$  vs group B:  $0.47 \pm 0.21$ ,  $P = 0.031$ ) (Table 3). There was no significant difference in atrophy scores between the two groups at biopsy site A1 and IA. group A showed significantly higher intestinal metaplasia scores than group B only at A1 (group A:  $2.10 \pm 0.41$  vs group B:  $1.12 \pm 0.29$ ,  $P = 0.035$ ), with no significant difference in intestinal metaplasia scores seen at all the other biopsy sites.



**Figure 3** Immunohistochemistry for *p53* (PAb240) in the *H pylori*-infected gastric mucosa. A small number of cells clearly showing the expression of *p53* (PAb240) were detected in the neck region before eradication. **A:** a 53-yr-old male with gastric ulcer; **B:** a 51-yr-old male with chronic gastritis;  $\times 400$ .

## DISCUSSION

Previously, we reported the significant over-expression of *p53* in the *H pylori*-infected gastric pits, as well as its significant reduction after *H pylori* eradication<sup>[18]</sup>. Other researchers also reported that *p53* was immunohistochemically detected in the non-neoplastic *H pylori*-infected gastric mucosa<sup>[24-28]</sup>, and that *H pylori* eradication therapy also diminished the expression of *p53*<sup>[18,25,27]</sup>.

*p53* is thought to be one major player in the regulation of cell cycle<sup>[29]</sup> in which DNA damage activates *p53* proliferation, and *p53* binds to MDM2 and other molecules. This binding protein arrests cell proliferation and maintains the integrity of the cell by DNA repairing or cell apoptosis. In contrast, mutant *p53* loses these abilities. In a previous report<sup>[18]</sup>, we considered that immunodetection of *p53* (DO-7) signified whether accelerated wild type-*p53* or occurrence of mutant type-*p53*. Teh *et al*<sup>[28]</sup> reported that *p53* detection in the non-neoplastic epithelium was considered to reflect an accumulation of wild-type *p53* due to the sensitivity of the currently available antigens. Our study also suggested an accumulation of wild-type *p53*, especially in the *H pylori*-infected mucosa probably because of the DNA damage that occurred to *H pylori* infection. In addition, the present study showed that quite a few cells were found positive for mutant-type *p53* (PAb240), instead of *p53* (DO-7). There has been no study reported to date demonstrating the positive immunohistochemical staining for mutant-type of *p53* in the non-neoplastic gastric mucosa. Our results indicated that while the majority of cells that reacted with *p53* (DO-7) account for an accumulation of wild-type *p53*, mutant type *p53* might also be expressed in some cells of the gastric pit. Murakami *et al*<sup>[14]</sup> and Morgan *et al*<sup>[31]</sup> reported the presence of point mutations in exon 5 to 8 of *p53* in *H pylori*-positive gastritis, and several other studies have shown that gastric precancerous lesions, such as atrophic gastritis and intestinal metaplasia, are associated with *p53* abnormalities<sup>[20-22,26, 32]</sup>. Murakami *et al*<sup>[14]</sup> reported that the point mutations are present in 52.4% of the gastritis, with Morgan *et al*<sup>[31]</sup> reporting the presence of *p53* mutations in 35% of the gastritis and 45% of the intestinal

**Table 1 Immunohistochemical detection against p53 (PAb 240) in gastric mucosa**

|                          | Case                     | Sex | Age (yr) | Clinical diagnosis   | Number of positive cells per ten crypts against p53 (PAb240) before eradication |     |      |     |     | Number of positive cells per ten crypts against p53 (PAb240) 6 mo after eradication |     |    |     |    |   |
|--------------------------|--------------------------|-----|----------|----------------------|---|-----|------|-----|-----|---|-----|----|-----|----|---|
|                          |                          |     |          |                      | A2  | A1  | IA   | B1  | B2  | A2  | A1  | IA | B1  | B2 |   |
| <i>H pylori</i> positive | 1                        | F   | 42       | Gstric ulcer         | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 2                        | F   | 26       | Duodenal ulcer       | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 3                        | M   | 64       | Gastric ulcer        | 0   | 0   | 11.4 | 0   | 0   | 0.7   | 0   | 0  | 0   | 0  | 0 |
|                          | 4                        | M   | 64       | Gastric ulcer        | 0   | 0   | 0    | 2.5 | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 5                        | M   | 56       | Gastric ulcer        | 0   | 0.8 | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 6                        | M   | 50       | Gastric ulcer        | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 7                        | M   | 45       | Gastroduodenal ulcer | 0   | 0   | 0    | 0   | 2.3 | 0   | 1.1 | 0  | 0   | 0  | 0 |
|                          | 8                        | M   | 52       | Chronic gastritis    | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 9                        | M   | 60       | Gastric ulcer        | 0   | 0   | 0    | 0.8 | 0   | 0.5   | 0   | 0  | 2.2 | 0  | 0 |
|                          | 10                       | F   | 71       | Chronic gastritis    | 1.7   | 0   | 0.8  | 0.7 | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 11                       | M   | 60       | Gastric ulcer        | 1.3   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 12                       | M   | 55       | Gastric ulcer        | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 13                       | M   | 42       | Gastroduodenal ulcer | 2.4   | 2.2 | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 14                       | M   | 68       | Gastric ulcer        | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 15                       | M   | 67       | Chronic gastritis    | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 16                       | M   | 57       | Chronic gastritis    | 0   | 0   | 0    | 2   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 17                       | F   | 77       | Gastric ulcer        | 0   | 0   | 0    | 1.7 | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 18                       | F   | 51       | Chronic gastritis    | 0   | 0   | 0    | 0.7 | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 19                       | M   | 64       | Gastric ulcer        | 0   | 0   | 0    | 0   | 2.3 | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 20                       | F   | 57       | Chronic gastritis    | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 21                       | M   | 51       | Chronic gastritis    | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 22                       | F   | 75       | Chronic gastritis    | 5.7   | 0   | 3.3  | 0   | 0.8 | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 23                       | F   | 65       | Chronic gastritis    | 0.5   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 24                       | F   | 51       | Chronic gastritis    | 0.6   | 0   | 2.7  | 0   | 4.2 | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 25                       | M   | 53       | Gastric ulcer        | 7.5   | 2.7 | 5    | 2.1 | 0   | 6   | 0   | 0  | 0.1 | 0  | 0 |
|                          | 26                       | M   | 68       | Chronic gastritis    | 0.9   | 2.9 | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 27                       | M   | 50       | Chronic gastritis    | 0   | 1.3 | 0.6  | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 28                       | F   | 78       | Chronic gastritis    | 0   | 0   | 0    | 2.2 | 1.8 | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 29                       | M   | 58       | Gastric ulcer        | 0   | 0   | 2.5  | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 30                       | M   | 74       | Chronic gastritis    | 0   | 0   | 0    | 1.7 | 4.5 | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 33                       | M   | 48       | Chronic gastritis    | 1.1   | 0   | 3.8  | 0   | 3.8 | 0   | 0   | 0  | 1.7 | 0  | 0 |
|                          | 34                       | F   | 68       | Chronic gastritis    | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 35                       | F   | 46       | Gastric ulcer        | 3.3   | 0   | 7.5  | 6   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 36                       | M   | 64       | Duodenal ulcer       | 0   | 0   | 0    | 6.7 | 0   | 2   | 1.7 | 0  | 0   | 0  | 5 |
|                          | 37                       | M   | 42       | Chronic gastritis    | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 38                       | F   | 71       | Chronic gastritis    | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 39                       | M   | 58       | Chronic gastritis    | 0   | 1   | 0.8  | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 40                       | F   | 46       | Chronic gastritis    | 0   | 0   | 0    | 0   | 0   | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 41                       | M   | 76       | Chronic gastritis    | 0.9   | 3   | 2.2  | 1.7 | 1.7 | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | 42                       | M   | 51       | Chronic gastritis    | 2.9   | 0   | 3.1  | 5.4 | 7.7 | 0   | 0   | 0  | 0   | 0  | 0 |
|                          | <i>H pylori</i> negative | 43  | M        | 25                   |   | 0   | 0    | 0   | 0   | 0   |     |    |     |    |   |
|                          |                          | 44  | M        | 49                   |   | 0   | 0    | 0   | 0   | 0   |     |    |     |    |   |
| 45                       |                          | M   | 51       |                      | 0   | 0   | 0    | 0   | 0   |   |     |    |     |    |   |
| 46                       |                          | M   | 68       |                      | 0   | 0   | 0    | 0   | 0   |   |     |    |     |    |   |
| 47                       |                          | M   | 40       |                      | 0   | 0   | 0    | 0   | 0   |   |     |    |     |    |   |
| 48                       |                          | F   | 64       |                      | 0   | 0   | 0    | 0   | 0   |   |     |    |     |    |   |
| 49                       |                          | F   | 52       |                      | 0   | 0   | 0    | 0   | 0   |   |     |    |     |    |   |
| 50                       |                          | M   | 59       |                      | 0   | 0   | 0    | 0   | 0   |   |     |    |     |    |   |
| 51                       |                          | M   | 73       |                      | 0   | 0   | 0    | 0   | 0   |   |     |    |     |    |   |
| 52                       |                          | F   | 59       |                      | 0   | 0   | 0    | 0   | 0   |   |     |    |     |    |   |
| 53                       |                          | M   | 38       |                      | 0   | 0   | 0    | 0   | 0   |   |     |    |     |    |   |

A1: Lesser curvature of the antrum; A2: Greater curvature of the antrum; IA: Lesser curvature of the angle; B1: Lesser curvature of the lower body; B2: Greater curvature of the upper body.

**Table 2 Positive ratio of Immunohistochemical detection against p53 (PAb 240)**

|                          | Before eradication   | 6 mo after eradication           |
|--------------------------|----------------------|----------------------------------|
| <i>H pylori</i> positive | 66.7% (28 out of 42) | 14.3% (6 out of 42) <sup>b</sup> |
| <i>H pylori</i> negative | 0% (0 out of 11)     |                                  |

<sup>b</sup>P < 0.01 vs before eradication in *H pylori* positive group.

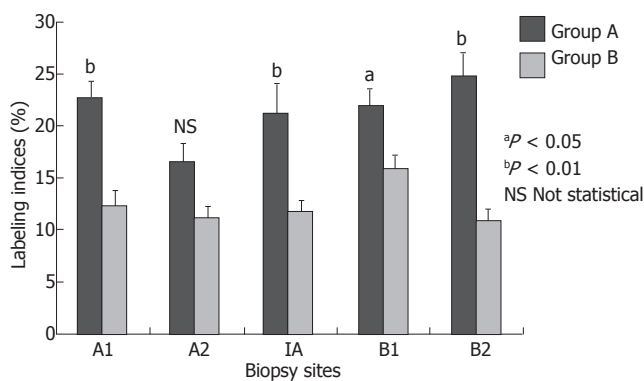
metaplasia. Unger *et al*<sup>[32]</sup> demonstrated that the expression of p53 and apoptotic indices in gastritis in the absence of intestinal metaplasia are significantly higher in *H pylori*-positive patients than in *H pylori*-negative patients. These studies are in agreement with our results indicating that in some p53 mutations are found mutant type-p53 products in the *H pylori*-infected gastric pits.

In a comparison between group A with more than six

**Table 3** The differences of updated Sydney system score between the group showed more than six positive cells for *p53* (PAb240) per 10 gastric pits (group A) and the group showed less than five cells for *p53* (PAb240) per 10 gastric pits (Group B)

| Contents of updated Sydney system | Group   | Biopsy points            |                          |             |                          |                          |
|-----------------------------------|---------|--------------------------|--------------------------|-------------|--------------------------|--------------------------|
|                                   |         | A2                       | A1                       | IA          | B1                       | B2                       |
| <i>H pylori</i>                   | Group A | 1.25 ± 0.25              | 0.50 ± 0.21              | 1.00 ± 0.21 | 0.88 ± 0.24              | 1.38 ± 0.21              |
|                                   | Group B | 0.71 ± 0.18              | 0.88 ± 0.22              | 0.88 ± 0.21 | 1.13 ± 0.19              | 1.41 ± 0.17              |
| Inflammation                      | Group A | 2.20 ± 0.12              | 2.20 ± 0.12              | 2.10 ± 0.16 | 2.30 ± 0.13              | 1.90 ± 0.16              |
|                                   | Group B | 2.05 ± 0.18              | 1.99 ± 0.17              | 2.00 ± 0.17 | 2.00 ± 0.12              | 1.82 ± 0.12              |
| Activity                          | Group A | 1.40 ± 0.15              | 1.00 ± 0.23              | 1.20 ± 0.23 | 1.20 ± 0.23              | 1.30 ± 0.23              |
|                                   | Group B | 1.06 ± 0.20              | 0.94 ± 0.18              | 1.00 ± 0.18 | 1.05 ± 0.16              | 0.94 ± 0.17              |
| Atrophy                           | Group A | 2.00 ± 0.14              | 1.75 ± 0.13              | 2.13 ± 0.28 | 2.00 ± 0.21              | 1.20 ± 0.30              |
|                                   | Group B | 1.40 ± 0.15 <sup>a</sup> | 1.57 ± 0.15              | 1.67 ± 0.18 | 1.07 ± 0.23 <sup>a</sup> | 0.47 ± 0.21 <sup>a</sup> |
| Intestinal etaplasia              | Group A | 0.90 ± 0.31              | 2.10 ± 0.41              | 1.80 ± 0.40 | 1.30 ± 0.36              | 0.20 ± 0.12              |
|                                   | Group B | 0.82 ± 0.27              | 1.12 ± 0.29 <sup>c</sup> | 1.47 ± 0.32 | 1.11 ± 0.29              | 0.12 ± 0.12              |

A1: the lesser curvature of the antrum; A2: the greater curvature of the antrum; IA: smaller curvature of the angle; B1: and the lesser curvature of the middle corpus; B2: the greater curvature of the upper corpus. <sup>a</sup>*P* < 0.05 vs atrophy scores in group A, <sup>c</sup>*P* < 0.05 vs intestinal metaplasia scores in group A.



**Figure 4** Labeling indices for *p53* (DO-7) in group A with more than 6 positive cells for *p53* (PAb240) per 10 gastric pits (*n* = 12) and group B with less than 5 positive cells for *p53* (PAb240) per 10 gastric pits (*n* = 30) before eradication. Results are shown as mean ± SEM. Labeling indices for *p53* (DO-7) were significantly higher for group A than for group B at all biopsy sites except for the lesser curvature of the antrum. <sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, NS, not statistical.

positive cells per 10 gastric pits and group B with less than five cells per 10 gastric pits, group A showed significantly higher labeling indices for *p53* (DO-7) in all biopsy sites than group B. This result suggested an increase in wild-type *p53* as it was caused by the greater DNA damage due to the gene instability in place that might increase mutant-type *p53*. Moreover, group A showed significantly higher atrophy and intestinal metaplasia scores in the stomach, suggesting that the gastric mucosa with more severe atrophic changes or extensive intestinal metaplasia has a greater number of mutant-type *p53*. Thus, it is likely that the development of atrophic changes and intestinal metaplasia gradually increased the occurrence of *p53* mutations. Gastric cancer of the intestinal type follows a multi-step process of carcinogenesis, such as atrophic gastritis, intestinal metaplasia, and dysplasia<sup>[33]</sup>. Therefore, the present results indicate that *H pylori* infection in the gastric mucosa may be implicated in the pathway of gastric carcinogenesis. Nardone *et al*<sup>[26]</sup> reported that DNA aneuploidy was seen in 11 patients with *H pylori* infection and atrophy, with 8 of these found to show c-Myc expression, and 6 of these to have *p53* expression, and they concluded that chronic *H pylori* infection may

be responsible for genomic instability in a subset of *H pylori*-positive chronic atrophic gastritis. Unger *et al*<sup>[32]</sup> also reported that *H pylori*-positive gastritis accompanied with intestinal metaplasia showed a lack of increased apoptosis with a higher *p53* expression, which suggests an increased genetic instability, thus concluding that *p53* mutation is an early step in the multi-step process of gastric carcinogenesis. Our results confirm the relationship between atrophic and/or metaplastic gastric mucosa with *H pylori* infection and genetic instability, which lead to gastric carcinogenesis.

In the present study, *H pylori* eradication therapy led to a significant reduction in the expression of both *p53* (DO-7) and *p53* (PAb240). The number of mutant-type *p53*-positive patients was significantly decreased 6 mo after eradication. In our previous study, we exhibited that eradication reduces both *p53* and MDM2 expression<sup>[18]</sup>. Hibi *et al*<sup>[25]</sup> and Satoh *et al*<sup>[27]</sup> also described a reduction in the expression of *p53* after eradication. Nardone *et al*<sup>[26]</sup> reported that *H pylori* eradication reduced gastritis activity, atrophy, and complete metaplasia, accompanied by the disappearance of markers of genomic instability, thereby concluding that eradication can reverse inflammation, associated atrophy, metaplasia, and genomic instability. The present study also indicated that *H pylori* eradication diminished *p53* abnormality, and this result accounted for the improvement of genetic instability.

Many researchers reported morphologic alterations of gastric mucosa, especially those of atrophic and metaplastic status after *H pylori* eradication<sup>[34]</sup>, while their results are conflicting, with some of them reporting that eradication did not improve atrophy and/or intestinal metaplasia, and some concluding that eradication could improve the gastric atrophy and/or intestinal metaplasia. However, after the year 2000, the majority of papers reported improvements in the gastric mucosa with *H pylori* eradication.

The present study confirmed the hypothesis that *H pylori* eradication reverses the atrophic changes in the gastric mucosa and the genetic instability, thus preventing the development of gastric cancer.

In conclusion, in chronic gastritis associated with *H pylori* infection, the expression of mutant-type *p53* was

seen significantly greater in more severe atrophic and metaplastic changes. *H pylori* eradication led to a significant reduction in the expression of mutant-type p53. It is suggested that chronic gastritis associated with *H pylori* infection has genetic instability, which leads to gastric carcinogenesis, and that *H pylori* eradication may prevent gastric cancer.

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