Immunohistochemical Detection of p53 and Serum Anti p53 Antibodies from Gastric Cancer Patients in Thailand

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ABSTRACT

Background & Aims: Gastric cancer is one of the most common malignancies worldwide. We performed to detect the correlation of various clinicopathological parameters with p53 expressions and serum anti p53 antibodies in Thai patients with gastric cancers.

Methods and Materials: Thirty eight patients with histological proven gastric cancer were recruited at King Chulalongkorn Memorial Hospital from 2001 to 2005. The tissues were collected for immunohistochemical detection of p53 and sera were analyzed for p53 antibodies using ELISA technique. The clinicopathological data were collected.

Results: Of 38 patients, there were 40.5% males and 59.9% females (mean age 63.48 ± 11.96 years). 6/8 (75.0%) of the positive p53 immunoreactivity patients had histological well to moderate differentiated adenocarcinoma, compared to 15/28 (53.6%) of those had histological poor differentiated adenocarcinoma or signet ring type. (p = 0.278). There were no relationships of p53 expression or serum anti p53 antibodies with clinicopathological data were collected including age, sex, symptom, presentation, history of cigarette smoking and alcohol drinking, lesion site, metastasis, treatment, H. pylori status, result of treatment, and survival time.

Conclusion: These results suggest that the expression of p53 or the detection of p53 antibodies has no significant impact on the outcome of Thai patients with gastric cancer.

Key words: p53, gastric cancer, Thailand

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INTRODUCTION

Gastric carcinoma is one of the most common malignant diseases worldwide and remains a significant problem in global health terms, with a wide variability in geographical distribution\(^{(1)}\). Gastric carcinogenesis is a complex and multifactorial process, in which many factors are implicated. The majority of gastric cancers are thought to be caused by environmental factors that result in damage to the mucosa and that inhibit its ability to repair itself\(^{(2)}\). *Helicobacter pylori* infection has been suggested to be a major risk factor for gastric carcinogenesis. Although *H. pylori* has been classified as a type I carcinogen for gastric cancer by the International Agency for Research on Cancer (IARC)\(^{(3)}\), the exact nature and strength of the association with gastric cancer has remained indistinct. Chronic *H. pylori* infection and dietary factors, such as those high in salt or nitrate, and nutritional deficiencies have been associated with gastric cancer\(^{(4)}\). This response is regulated, in part, by inhibitory and stimulatory factors that are products of proto-oncogenes and tumor suppressor genes\(^{(5)}\).

It has been suggested that p53 gene mutations are responsible for gastric carcinogenesis\(^{(6,7)}\). The frequency of tumors with p53 expression in gastric carcinoma has been reported to vary from 4% to 71%\(^{(8-10)}\). It has been reported that accumulation of p53 is found in gastric mucosa infected with *H. pylori* in adults and decreased after eradication therapy of *H. pylori*\(^{(11,12)}\). The p53 Ab is an autoantibody induced by mutation of the p53 tumor suppressor gene, and has been detected in the sera of patients with various types of cancers\(^{(13,14)}\).

The prognostic value of p53 in gastric cancer is still unclear\(^{(15,16)}\). In this study, p53 expressions were investigated by immunohistochemistry and serum anti p53 antibodies were detected for the correlation with various clinicopathological parameters in Thai patients with gastric cancers.

PATIENTS AND METHODS

Thirty eight patients who underwent esophagogastrroduodenoscopy, with histological proven gastric cancer were recruited from GI unit, King Chulalongkorn Memorial Hospital from 2001 to 2005. The Ethical Committee of the Faculty of Medicine, Chulalongkorn University, approved the study. Informed consents were obtained from all patients. The tissue samples were collected. Samples were stored at -80°C until they were assayed. The clinicopathological data were collected including age, sex, symptom, presentation, history of cigarette smoking and alcohol drinking, lesion site, histological type, metastasis, treatment, *H. pylori* status, blood chemistry, result of treatment, and survival time. Detection of *H. pylori* was diagnosed by positive rapid urease test or histological examination.

Immunohistochemistry

Sections from each representative specimen were cut at 3-5 mm, mounted on glass and dried overnight at 37 °C. Briefly, all sections were then deparaffinized in xylene, dehydrated through a graded series of alcohol and washed in distillated water. Distillated water was used for all subsequent washes and dilution of the antibodies. Endogenous peroxidase activity was blocked using a 0.3% solution of hydrogen peroxidase. Tissue sections were put in citrate buffer pH 6.0 in high pressure cooker. Monoclonal mouse anti-human p53 protein antibody (Clone DO-7, Neomarkers, Inc. 47790 Westinghouse Dr., Fremont CA 94539, USA) at a 1/50 dilution, monoclonal mouse anti-human antibody anti-Bcl-2 (Clone 7D9, Neomarkers, Inc. 47790 Westinghouse Dr., Fremont CA 94539, USA) at a 1/50 dilution, and Dako En Vision labeled polymer-HRP (anti mouse). Diaminobenzidine was used as the final chromogen, and Mayer’s haematoxylin as the nuclear counter stain.

Serum anti p53 antibodies-ELISA

Serum p53-Ab levels were assessed by using ELISA kits (quantikine R & D systems, Inc., Minneapolis, MN) according to the manufacturer’s instructions. Briefly, the samples were added, for 1h at 37 °C, to microtiter wells coated with wild-type human p53 protein or a control protein to detect nonspecific interactions. After washing, a peroxidase-conjugated monoclonal antibody directed to a second epitope was added. Then substrate solution was added for 30 min at 37 °C. The color reaction was terminated by a stop solution containing sulfuric acid, and absorbance was measured at 450 nm, using a spectrophotometer. Results were calculated from a standard curve generated by a four-parameter logistic curve-fit and expressed in pg/mL.

Statistical analysis

SPSS software (version 11.00) was used for statistical analysis. Data are expressed as percentage,
mean and standard deviation. Comparisons between groups were analyzed by the Chi-square test and Fisher’s exact test for categorical variables and Student’s t test for quantitative variables. $P < 0.05$ was taken as statistically significant.

**RESULTS**

Of 38 patients with gastric cancer, there were 40.5% males and 59.9% females with ages ranging from 40 to 85 years (mean age 63.48 ± 11.96 years). The common presentations were dyspepsia (41.2%), weight loss (39.2%), and upper gastrointestinal bleeding (15.7%), respectively. Almost lesions were located in pyloric-antrum and greater curvature of gastric body. *H. pylori* infection was detected in 25% of patients.

**p53 Immunohistochemistry**

According to the classification by Lauren, stomach cancer is classified into two main histological types: diffuse and intestinal. The detection of p53 positively in intestinal-type carcinomas is not differ from in diffuse-type carcinomas (56% vs 36%, $p = 0.471$).

For the tumor differentiation; there were 10.5% well differentiated, 15.8% moderate differentiated, 44.7% poorly differentiated and 29.0% signet ring type, respectively. The correlation of tumor differentiation and p53 immunoreactivity was shown in Figure 1 and Figure 2. 6/8 (75.0%) of the positive p53 immunoreactivity patients had histological well to moderate differentiated adenocarcinoma, compared to 15/28 (53.6%) of those had histological poor differentiated adenocarcinoma or signet ring type. ($p = 0.278$).

There were no relationships of p53 expression with clinicopathological data were collected including age, sex, symptom, presentation, history of cigarette smoking and alcohol drinking, lesion site, metastasis, treatment, *H. pylori* status, result of treatment, and survival time.

**Serum anti p53 antibodies**

Levels of p53-Abs were determined form a calibration curve constructed from the specific signals of standards, using a p53 index of 1.7 pg/mL as the cut-off value, 9/27 patients with gastric carcinoma were positive for serum p53 antibodies (33.3%). There were no relationships of p53 antibodies with all clinicopathological data. Moreover, there was no statistical difference between the p53 Abs-positive and p53 Abs-negative groups with *H. pylori* status, as shown in Table 1.

![Figure 1. The correlation of tumor differentiation and p53 immunoreactivity](image-url)
**DISCUSSION**

Gastric carcinoma is one of the most frequently occurring cancers, and is considered to be the fifth most common malignant and the second leading cause of cancer death in the world\(^\text{17}\). Gastric carcinogenesis is a complex, multistep, and multifactorial process, in which many factors are implicated. *Helicobacter pylori* is a major contributory factor in the development of human gastric cancer. It is classified as a group 1 carcinogen by the World Health Organization\(^\text{18}\) but the exact nature and strength of the association with gastric cancer has remained indistinct. Increased p53 accumulation has been previously reported in the gastric mucosa infected with *H. pylori\(^\text{6}\). Jones et al. showed that p53 expression index was significantly higher in patients with *H. pylori* positive gastritis than in those with *H. pylori* negative gastritis and those without gastritis\(^\text{11}\). Murakami et al. reported point mutations in p53 exon 7 and 8 by direct sequence analysis in *H. pylori* infected gastritis, but not in *H. pylori*-uninfected gastric mucosa\(^\text{19}\). These reports suggest that the increased levels of p53 protein detected in the neck region may represent the accumulation of a mutant p53 type.

The p53 gene is located at chromosome 17p13.1, encodes a 53kDa nuclear phosphoprotein (p53 protein)\(^\text{20}\). The p53 protein is a DNA-binding, oligomerization domain and transcription activation domain-containing a tumor suppressor that up-regulates growth arrest and apoptosis-related genes in response to stress signals, thereby influencing programmed cell death, cell differentiation and cell cycle control mechanisms\(^\text{21,22}\). The tumor suppressor gene now widely researched because it mutates in about 60-70% of most types of human cancers arising from a wild spectrum.
By immunohistochemistry, p53 protein overexpression is detected in many human neoplasms such as gastric, colon, and lung carcinomas. The prognostic value of p53 in gastric cancer is still unclear. The p53 mutation is accepted as an early event in gastric carcinomas. The increased p53 expression and gene mutations have also been reported in gastric premalignant mucosa, such as dysplasia, atrophy or even mucosa without obvious abnormality, suggesting that p53 function is affected from the early stage of gastric carcinogenesis. In some study, p53 expressions were investigated by immunohistochemistry and correlated with various clinicopathological parameters and survival. A correlation between p53 expression and the presence of metastasis was noted by Kakeji et al. However, some studies have shown no relationship of p53 expression with liver metastasis or lymph node involvement. In Deveci MS et al. study found the presence of p53 expression in early carcinomas, in tumors of 3 cm or less, and in patients with a lymphocytic response, as well as the absence of a relationship between p53 expression and lymph node metastases, suggest that p53 positivity could favor an early event in gastric carcinomas. In Starzynska T et al. series, the p53 overexpression correlated significantly with some clinicopathological features included; age younger than 60 years and tumor size more than 5 cm with a statistical trend towards significance with lymph node involvement. In our study, we did not observe a significant association between p53 expression and clinicopathological data although what has been reported earlier. We only presented that we found the more positive p53 immunoreactivity in patients who had histological well to moderate differentiated adenocarcinoma than who had histological poor differentiated adenocarcinoma or signet ring type (75.0% vs 53.6%, \( p = 0.278 \)).

Mutational p53 gene encodes mutational p53 protein whose half-life is longer than wild p53 protein's. The protraction of the half-life could lead to the overexpression of the p53 protein in cells, and it may lead to the production of the anti-p53 antibodies (p53 Abs) in serum. ELISA assisted by the recombinant p53 protein is a simple and reliable method to detect p53 Abs in serum. Circulating p53 Abs in patients have been reported for various types of carcinomas, including breast cancer, hematopoietic malignancy, esophageal cancer, colon cancer, ovarian cancer, lung cancer, pancreatic cancer, and gastric cancer. The p53 Abs have been reported to be positive in 11.2%-20.3% of patients with gastric carcinoma. In this study, we found 33.3% of patients with gastric carcinoma were positive for serum p53 antibodies. Previous reports indicated that the p53 Abs may be the prognosis marker of gastric carcinoma and showed the correlation between p53 Abs of patients with gastric carcinoma and lymph node metastasis, tumor stage. Because alterations in the p53 gene result in an accumulation of the protein in tumor cells, the presence of serum p53 Ab was described as an early event that could predate the diagnosis. In this study, the cutoff value for serum p53 Abs was 1.7 pg/mL. The specificity of this assay is greater than 95.5%. We could not indicate the p53 Abs correlated with the clinicopathological data including; age, sex, serum albumin, hematocrit level, cell differentiation, metastasis, and \( H. pylori \) status.

### CONCLUSIONS

At present, immunohistochemistry of p53 protein overexpression and serum anti p53 antibodies is de-
tected from Thai gastric cancer patients. Although there is no satisfactory marker for the clinicopathological correlation, it is expected that more data will be established.

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