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Original Paper

Presence of Poorly Differentiated Component Correlated with Submucosal Invasion in the Early Diffuse-type Gastric Cancer

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ABBREVIATIONS: HK, anti-H+, K+-ATPase antibody; Pepsinogen (PG); Helicobacter pylori (H. pylori); Hematoxylin and Eosin (HE)

ABSTRACT

Background/Aims. Diffuse-type gastric carcinoma is associated with a poor prognosis. However, the clinical behavior of diffuse-type gastric cancer is not fully understood. The aim of this study is to distinguish the behaviors of early diffuse-type gastric carcinomas by sub classifying tumors according to their histologic features.

Methodology. A total of 114 cases of diffuse-type early gastric cancer were studied retrospectively. We analyzed and compared the resected cancer specimens according to the histologic components: as poorly differentiated adenocarcinoma component-present (poor+) versus poorly differentiated adenocarcinoma component-absent (poor-). Helicobacter pylori status was evaluated by Giemsa staining and IgG serology. We assessed the degree of cancer invasion and compared background status of gastritis in accordance with the updated Sydney System criteria and serologic markers.

Results. In comparison to the poor+ cancers, the poor- cancers had a significantly larger portion of cells confined to the mucosa (p=0.002). Only 8 of the 114 cases were regarded as H. pylori-negative. Although we could not detect any serologic markers specific for gastritis with poor+ cancer, but the serum levels of gastrin was slightly higher in patients with poor+ cancers than in those with poor- cancers.

Conclusions. The biologic behavior of poor+ gastric carcinoma is worse than that of poor- carcinoma. There is a close relation between H. pylori infection and carcinogenesis of poorly differentiated adenocarcinoma.

INTRODUCTION

The mechanism underlying development of gastric adenocarcinoma, the most common form of gastric cancer, is not fully understood. However, infection by Helicobacter pylori is regarded as a trigger for gastric carcinogenesis (1-3). There is strong evidence for H. pylori infection as a cause of chronic atrophic gastritis and intestinal metaplasia, two possible precancerous lesions (4). Lauren classified gastric adenocarcinoma into two histologic types, intestinal and diffuse, according to the morphologic features of the tumors (5). Recent studies have indicated that H. pylori infection is associated with both types of gastric cancer (6,7).

Diffuse-type gastric carcinoma, generally associated with a poor prognosis, consists of one or more types of cancer cell components (signet-ring cell carcinoma, poorly differentiated adenocarcinoma, moderately differentiated tubular adenocarcinoma). Advanced diffuse-type gastric carcinoma is characterized by the potential to infiltrate diffusely into the gastric wall, but researchers have reported a significantly better outcome in cases of early gastric carcinoma with the presence of signet-ring cells than in cases of early gastric carcinoma with the presence of poorly differentiated adenocarcinoma (8,9). Ishiguro *et al.* reported that the histologic progression of differentiated-type to undifferentiated-type carcinoma takes place in the

Hepato-Gastroenterology 2008; 55:2264-2268

mucosa (10). However, little is known about the carcinogenic pathway or clinical aspects of the various diffuse-type gastric carcinomas including differences in the background gastric mucosa.

In the present study, we sub classified early diffuse-type gastric carcinomas according to histologic features, then compare and tried to clarify the biologic behaviors of early diffuse-type gastric carcinomas. Our aim is to distinguish patients for whom prognosis is good from those for whom prognosis is poor within the patients with diffuse-type gastric cancer. Those with a good prognosis can then be singled out for less invasive surgery. Moreover, we also analyzed and compared the background gastritis by histologic and serologic markers to identify high-risk group of cancer patients with poorer prognosis.

METHODOLOGY

Tissue samples

Tissue samples were obtained from 114 patients with informed consent. They had undergone gastrectomy or endoscopic mucosal resection for diffuse-type gastric cancer. The patients comprised 60 men and 54 women with a mean age of 58.0 years (range 22-86 years) who were treated at Hiroshima University Hospital between 1991 and 2004.

Histologically, cancer cells were confirmed to be limited to the submucosa in all cases. We assessed the histologic status of gastritis in specimens from the lesser curvature of the gastric antrum and the corpus. Hematoxylin and eosin (HE)-stained sections of gastric biopsy samples or resected tissues were examined in accordance with the criteria of the updated Sydney System as follows: for inflammation, i.e., severity of lymphocyte and plasma cell infiltration in the lamina propria; for activity, i.e., density of neutrophil infiltration; for atrophy, i.e., degree of loss of fundic and pyloric glands; and for intestinal metaplasia (11). According to the updated Sydney System, scoring was on a scale of 0 to 3 for each item in the antrum and the corpus mucosae. Early gastric cancer, that is, cancer limited to the submucosal layer, was diagnosed according to the pathologic criteria of the Japanese Research Society for Gastric Cancer (12). The research protocol was approved by the ethics committee at Hiroshima University Hospital.

Subclassification of gastric cancers

We analyzed and compared the resected cancer specimens in two different ways according to the histologic components: as poorly differentiated adenocarcinoma component-present (poor+) versus poorly differentiated adenocarcinoma component-absent (poor-) and as moderately differentiated tubular adenocarcinoma component-present (tub+) versus moderately differentiated tubular adenocarcinoma component-absent (tub-).

Assessment of Helicobacter pylori infection

H. pylori infection was evaluated in all cases with an anti-H. pylori antibody (E-plate, Eiken Chemical Co. Ltd., Tokyo, Japan) and by histologic examination of Giemsa-stained sections. Samples were regarded as H. pylori-negative when both tests were negative.

Measurement of serum pepsinogen and gastrin

Fasting serum was collected from each patient upon entry into the study. The sample was centrifuged immediately at 4°C and stored at -20°C until use. Serum concentrations of pepsinogens (PGs) and gastrin were determined by modified radioimmunoassay (13).

Preparation of antigens and enzyme-linked immunosorbent assay (ELISA)

H^+, K^+-ATPase was prepared from pig gastric mucosa. Vesicular membranes enriched with H^+, K^+-ATPase were treated with a low concentration of detergent (0.13% (w/v) sodium n-octylglucoside or 0.06% (w/v) sodium dodecylsulfate) to remove loosely attached proteins and then stored at -70°C in sucrose /HEPES-Tris buffer, pH 7.4. Multi-well plates were covered with 50 ml of the indicated antigen preparations (5 mg/ml) in 50 mmol/l Na_2CO_3-$NaHCO_3$ buffer, pH 9.8, and incubated overnight at 4°C. Incubation was with sera diluted 1:100 in phosphate-buffered saline containing 0.05% (v/v) Tween 20 (PBS-T), biotinylated goat anti-human IgG (Amersham, Buckinghamshire, UK), streptavidin (Amersham), and biotinylated alkaline phosphatase (Boehringer-Mannheim Biochemicals, Mannheim, Germany). Finally, 100 μl p-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO, USA) at 1 mg/ml in 50 mmol/l Na_2CO_3-$NaHCO_3$ buffer, pH 9.8, was added. The absorbance was read continuously at 405 nm (kinetic ELISA) with a computerized ELISA reader (Vmax®, Molecular Devices, Sunnyvale, CA, USA). All incubations were performed with continuous shaking, and the plates were washed three times with PBS-T between steps. Positive and negative control standards were included in each plate. The optical density (mod per min) for each sample was normalized to the positive standard on each plate, and the data are presented as relative antibody titers. The coefficients of interassay variation (calculated as 100 x SD/mean) of the positive and negative H^+, K^+-ATPase-ELISA standard were < 5 and < 13 (14).

Statistical analysis

Results are reported as the mean±SD. We used the chi-square test, Student's unpaired t-test, multivariate logistic regression analysis, or Mann-Whitney U test where appropriate. Results were considered statistically significant when p values were less than 0.05.

RESULTS

Histologic results

The overall study group and histopathologic types of diffuse gastric cancer are shown in **Table 1**. Poorly differentiated components were absent in 44 cancers in which the histologic features comprised only signet-ring cell carcinoma or signet-ring cell carcinoma and moderately differentiated adenocarcinoma. Moderately

TABLE 1 Study Group-Clinicopathologic Data

Number of cases	114
Sex ratio (Male/Female)	60/54
Mean age (range)	58.0 (22 - 86)
Histologic type of gastric cancer	
poor+/poor-	70/44
tub+/tub-	40/74

Poor+, poorly differentiated adenocarcinoma is present;
Poor-, poorly differentiated adenocarcinoma is absent;
Tub+, moderately differentiated tubular adenocarcinoma is present;
Tub-, moderately differentiated tubular adenocarcinoma is absent.

TABLE 2 Multivariate Logistic Regression Analysis of the Risk Factors for Submucosal Invasion

	Odds ratio (95% CI)	*p* value
poor+ v poor-	4.7 (1.5-14.6)	0.007*
ub+ v tub-	1.1 (0.43-2.8)	0.85

CI: Confidencial Interval; Poor+: poorly differentiated adenocarcinoma is present; Poor-: poorly differentiated adenocarcinoma is absent; Tub+: moderately differentiated tubular adenocarcinoma is present; Tub-: moderately differentiated tubular adenocarcinoma is absent.;
*Statistically significant

differentiated tubular adenocarcinoma components were absent in 74 cancers in which the histologic features comprised only poorly differentiated adenocarcinoma cell, only signet-ring cell carcinoma or poorly differentiated adenocarcinoma cell and signet-ring cell carcinoma. Multivariate logistic regression analysis was performed for the correlation between tumor invasion to submucosal layer and histologic features. The poor+ cancers were significantly correlated with the tumor invasion to submucosal layer (**Table 2**). We compared the depth of tumor invasion between poor+ and poor- cancers. The poor- cancers in comparison to the poor+ cancers had a signifantly larger portion of mucosa-confined tumor cells (p=0.002; **Table 3**).

Helicobacter pylori infection and histologic features

We evaluated the status of H. pylori infection in all cases. Only 8 of the 114 cases were H. pylori-negative. Five of the H. pylori-negative cancers were poor- cancers (**Table 4**). The rate of H. pylori infection was slightly higher in patients with poor+ cancers than in those with poor- cancers, but no statistical differences were found.

Background status of gastric mucosa evaluated by histological examination and by serologic markers

Next, we attempted to identify histologic differences in gastritis between poor+ and poor- cancers with H. pylori infection. Mean scores of gastritis are shown in **Table 5**. There was no significant difference between them in atrophy, mononuclear cell infiltration, neutrophil infiltration, and intestinal metaplasia. We selected some serologic markers of gastritis (PG-I, PG-II, PG-I/II, gastrin, and anti- H+,K+-ATPase antibody (HK)) and compared titers between cancer types. As shown in **Table 6**, no differences in PG-I, PG-II and HK were detected in poor+ cancers compared to levels in poor- cancers. Serum levels of gastrin was slightly higher in patients with poor+ cancers than in those with poor- cancers, but no statistical differences were found.

TABLE 3 Depth of Invasion according to Histologic Types of Gastric Cancer

Histologic type	Depth of invasion		*p* value†
	Mucosa	Submucosa	
poor+	43 (61.4%)	27 (38.6%)	0.002*
poor-	39 (88.6%)	5 (11.4%)	

Poor+: poorly differentiated adenocarcinoma is present; Poor-: poorly differentiated adenocarcinoma is absent; †Chi-square test.
*Statistically significant.

TABLE 4 H. pylori Infection Status according to Pathologic Subclassifications

Histologic type	H. pylori-negative	H. pylori-positive	*p* value†
poor+	3 (4.3%)	67 (95.7%)	0.26
poor-	5 (11.4%)	39 (88.6%)	

Poor+: poorly differentiated adenocarcinoma is present; Poor-: poorly differentiated adenocarcinoma is absent; †Chi-square test.

DISCUSSION

Gastric cancer is generally classified into two types: the intestinal type and the diffuse type. This classification system is widely accepted and is clinically important, for example, in the criteria for endoscopic mucosal resection. Among the early gastric cancers in our study (defined as malignant tumor confined to the mucosa and submucosa regardless of the presence or absence of metastasis to the perigastric lymph nodes), the poorly differentiated-absent (poor-) cancers, in comparison to the poorly differentiated-present (poor+) cancers, had a significantly larger portion of tumor cells confined to the mucosa. This means the prognosis for patients with poor+ cancer is worse than that for patients with poor- cancers. Similar results were reported by Hyung *et al*, who defined groups according to the predominant cancer cell component (8). Because signet-ring cells, in comparison to poorly differentiated adenocarcinoma cells, have less ability to invade the submucosa, signet-ring cell carcinoma limited within the mucosal layer may be regarded as an indicator for endoscopic mucosal resection of tumors with clear lateral borders. The reason for this difference between poor+ and poor- cancers is unclear, but the expression patterns of some factors, such as growth factors, angiogenic factors, or proteolytic enzymes may differ between the two types.

It is important to investigate the background gastritis status not only with respect to the pathogenesis of gastric cancer but also with respect to cancer screening. Because atrophic changes are not severe in diffuse-type gastric cancer, such cancer was previously believed to have little relation to H. pylori infection (15-16).

However, epidemiologic and histopathologic studies have indicated that H. pylori infection is associated with both the intestinal-type and diffuse-type of gastric cancer (7). Of the 114 cases of diffuse-type gastric cancer in our study, 106 (93%) were H. pylori-positive. Almost all patients with poor+ cancer showed H. pylori infection, suggesting a close relation between H. pylori infection and the development of poorly differentiated cancer, which has a strong tendency to invade into submucosal layer. We reported that H. pylori infection upregulated the ability of angiogenesis and invasion of the TMK-1 cell line which was established from a poorly differentiated adenocarcinoma (17). H. pylori infection may also regulate angiogenesis and invasion of poorly differentiated gastric cancer in vivo.

Patients with poorly differentiated cancer may have a poor prognosis. It is therefore important to clarify the background gastritic status of these patients. Yanagisawa *et al.* reported that signet-ring cell carcinomas were surrounded by fundic gland mucosa without intestinal metaplasia or pseudopyloric glands, whereas poorly differentiated adenocarcinomas were accompanied by at least one of these changes (18). Although, we attempted to identify histologic or serologic markers specific for poor+ cancer, we found no differences in background gastric mucosa status between poor+ and poor- cancer. We found a tendency toward relatively high serum levels of gastrin in patients with poorly differentiated cancers. Gastrin secretion is modulated by many factors, including luminal pH and vagal cholinergic regulation (19). When H. pylori infection involves the oxintic mucosa, leading to atrophy of the oxyntic glands and accompanying reduced gastric acid secretion and hypergastrinemia (20). H. pylori infection also induces gastric inflammatory cytokines, such as interluikin IL-1, IL-6, IL-8, and tumor necrosis factor-α (TNF- α) (21), which stimulate the G-cell to produce gastrin (22-24). In our study, there was no difference between poor+ and poor- cancers in atrophy and serum levels of PG-I, PG-II and anti-HK. H. pylori infection with the poor+ cancers may induce cytokines which cause hypergastrinemia. In consideration of our results, gastrin may has a relation with the occurrence of the poorly differentiated adenocarcinoma component in part. Inhibitors of gastric acid secretion are often used in the treatment of peptic ulcer. Moreover, such drugs are prevalently used to treat patients with gastroesophageal reflux disease (GERD) (25). The risk of treatment with these drugs should be verified in the view of this point.

TABLE 5 Histologic Findings of Gastric Mucosa in Two Groups

	Poor+ (n=56)	Poor- (n=36)	P value†
Atrophy			
Corpus	1.63 ± 0.93	1.36 ± 1.11	0.28
Antrum	1.83 ± 0.70	1.63 ± 0.89	0.38
Mononuclear cell infiltration			
Corpus	1.54 ± 0.63	1.42 ± 0.66	0.53
Antrum	1.41 ± 0.62	1.35 ± 0.67	0.89
Neutrophil infiltration			
Corpus	0.61 ± 0.65	0.61 ± 0.50	0.79
Antrum	0.36 ± 0.55	0.41 ± 0.57	0.71
Intestinal metaplasia			
Corpus	1.05 ± 1.03	0.91 ± 1.10	0.48
Antrum	1.22 ± 1.04	0.93 ± 1.09	0.29

Poor+: poorly differentiated adenocarcinoma is present; Poor-: poorly differentiated adenocarcinoma is absent; †Mann-Whitney U test.

TABLE 6 Serologic Markers for Gastric Inflammation in Two Groups

	Poor+ (n=29)	Poor- (n=24)	P value
PG I (ng/ml)	55.5 ± 32.5	58.1 ± 30.1	0.76
PG II (ng/ml)	22.8 ± 14.0	25.2 ± 17.7	0.59
PG I /PG II	2.54 ± 1.38	2.73 ± 1.29	0.62
High PG † /Low PG	13/16	14/10	0.33
Gastrin (pg/ml)	247 ± 223	165 ± 108	0.10
HK (relative titer)	6.48 ± 7.43	4.37 ± 3.48	0.40

† High PG: PG I >70 ng/ml or PG I /PG II >3.0

Poor+: poorly differentiated adenocarcinoma is present;
Poor-: poorly differentiated adenocarcinoma is absent.

A limitation of our study was the histopathologic evaluation of gastritis in gastric specimens only from the limited portions of lesser curvature to determine the condition of the background gastric mucosa. Moreover, a more detailed investigation of increased numbers of patients may be necessary to identify histologic or serologic differences.

In conclusion, we identified two subclasses of diffuse-type gastric cancer for which the clinical behavior differed. The clinical behavior of poor+ cancer appears to be worse than that of cancers with poor- cancer, and poorly differentiated cancers appear to be strongly related to H. pylori infection. Further study will focus on characterization of the bacterial factors that cause poorly differentiated cancer.

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