Background Submucosal Cysts in Early Gastric Cancer Cases Have Unique Clinicopathologic Features Suggestive of Postgastritis and Significant Smoking Association

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Key Words: Submucosal cyst; Gastric cancer; Gastritis; Smoking; Helicobacter pylori

Abstract

Submucosal cysts (SMCs) might result from severe gastritis and be related to gastric carcinogenesis, although direct evidence is limited. We studied clinicopathologic findings for gastric cancers arising in mucosa with SMC and the relation to gastritis. In 504 submucosal invasive cancer cases, SMC was found in 100. Comparison of degrees of gastritis using the Updated Sydney system, thickness of muscularis mucosae, and the patients' smoking and drinking habits and obesity showed significant variation between cases of cancer with and without SMC. In the stomach with SMCs, cancers were predominantly differentiated-type adenocarcinomas in men and showed a significant tendency for location in the upper gastric region. Intestinal metaplasia was significantly more severe and the muscularis mucosae were thicker in cancer cases with SMC in comparison with cases without SMC and control cases of gastrointestinal stromal tumor (GIST). Atrophy was also significantly more severe in cancer cases with and without SMC than in cases of GIST. The Brinkman index was also significantly higher. Cases of gastric cancer with SMC show characteristic clinicopathologic features, and SMC formation may be caused by gastritis and influenced by smoking.

Materials and Methods

A total of 504 cases of early gastric cancer with submucosal invasion in which patients underwent surgery between June 1986 and April 2003 at Kitasato University East
Hospital, Sagamihara, Japan, were reviewed. All of the resected materials were fixed in 10% buffered formalin, and entire lesions were step-sliced at 3 mm and embedded in paraffin. We cut 4-µm-thick sections and used them for H&E staining. Following the recommendations of the Japanese Research Society for Gastric Cancer,7 not only cancer lesions but also background mucosa of the lesser and greater curvatures were routinely examined for their histologic features. Histopathologic classification was based on predominant patterns of tumors. Well- to moderately differentiated adenocarcinomas were grouped together as differentiated adenocarcinomas for comparison with poorly differentiated adenocarcinomas and signet-ring cell carcinomas, defined as undifferentiated adenocarcinomas.8,9 The median number of sections was 23 per case. Clinical data were checked by using clinical charts.

Two pathologists (M.I. and T.M.) reviewed all specimens and assessed the distribution and degree of SMC development in the background gastric mucosa. In the 504 cases, at least 1 focus of SMC was confirmed in 100. Clinicopathologic data are summarized in Table 1, and findings for the distribution of SMC are illustrated in Figure 1. There was no significant variation in tumors in the upper, middle, and lower parts of the stomach, but SMCs were found at particularly high frequency in cases of cancer occurring in the upper part of the stomach. Therefore, cancer cases of the upper part of the stomach were further examined in this study, 31 with SMC and 35 without SMC being randomly selected. In addition, 27 gastrointestinal stromal tumor (GIST) cases without carcinoma were examined as control cases.

### Table 1

<table>
<thead>
<tr>
<th>Clinical Pathologic Characteristics of 504 Early Gastric Carcinoma Cases With or Without SMCs</th>
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<tbody>
<tr>
<td>Carcinoma With SMC</td>
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<tr>
<td>Total cases</td>
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<td>Median age (range, y)</td>
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<td>Sex</td>
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<td>Male</td>
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<td>Female</td>
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<td>Maximum mean ± SD tumor diameter (cm)</td>
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<td>Tumor location in stomach</td>
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<td>Upper part</td>
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<td>Lower part</td>
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<tr>
<td>Histologic type§,†</td>
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<tr>
<td>Differentiated</td>
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<td>Undifferentiated</td>
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</table>

SMC, submucosal cyst.

Data are given as number (percentage) unless otherwise indicated.

§ P < .1408.

† P < .0001.

‡ P < .0945.

| Evaluation of Gastritis in Background Mucosa |

The grade of gastritis was evaluated according to the Updated Sydney System using H&E-stained sections.10 The factors with this system are as follows: *H pylori* infection, neutrophil infiltration, chronic inflammation, glandular atrophy, and intestinal metaplasia. Each factor is scored for 4 degrees: normal, mild, moderate, and marked. We studied 3 stomach portions in each case: A1, the lesser curvature of the antrum; B1, the lesser curvature of the corpus, about 4 cm proximal to the angulus; and B2, the middle portion of the greater curvature of the corpus, about 8 cm from the cardia.

| Evaluation of *H pylori* Infection |

Histologic assessment for *H pylori* was performed using 4-µm-thick histologic sections with rapid Romanowsky (modified Giemsa) staining and immunostaining using a streptavidin-biotin-immunoperoxidase complex method. Briefly, for immunostaining, sections from the 3 aforementioned portions were deparaffinized and heated in citrate buffer solution (0.01 mol/L; pH 6.0) for 15 minutes using a microwave oven to retrieve antigens. As the primary antibody, an anti-*H pylori* antibody (polyclonal, ×50 dilution; DakoCytomation, Copenhagen, Denmark) was used. The sections were processed using an LSAB kit (DakoCytomation) according to the manufacturer’s manual, with 3, 3'-diaminobenzidine as the chromogen. Under high-power magnification (×400), spiral-shaped rods in the mucous layer of the surface epithelium and on the foveolar epithelium were confirmed to be *H pylori* by rapid Romanowsky and immunohistochemical staining.

| Thickness of the Muscularis Mucosae |

The thickness of the muscularis mucosae was measured on H&E-stained slides by using Digital Binocular Filar Micrometer Measuring Equipment A2 (Kogaku, Osaka, Japan). To ensure measurement of the full thickness of the...
muscularis mucosae, only points in which the longitudinal lumen consisting of the foveolar epithelium was fully identifiable were examined. Five points per region were measured, and the thickness was expressed as a median value.

Assessment of Patient Smoking and Drinking Habits and Obesity

Reviewing the clinical data, we studied a total of 66 cases for smoking and drinking habits and the body mass index (BMI; kg/m²) to evaluate obesity. Smoking exposure was expressed by using the Brinkman index and drinking habits as the amount of alcohol consumption (g/d). Current drinking habits and drinking history were checked for 5 alcoholic beverages: sake, shochu, beer, whisky and brandy, and wine. If the consumption was limited to certain seasons, the frequency and the amount were specified for the seasons. Ethanol intake was estimated from the frequency and amount of consumption of each beverage by using the approximate volume concentrations of ethanol (sake, 16%; shochu, 25%; beer, 4.5%; whisky, 40%; and wine, 12%).

Statistical Analysis

Data are expressed as median (range) or mean (± SD) values. Comparisons between groups were made with the χ² or Mann-Whitney U tests as appropriate. Statview software (Abacus Concepts, Berkeley, CA) was used for all statistical analyses, and a P value of less than .05 was considered to indicate statistical significance.

Results

Clinicopathologic Findings

Of the 504 cases of gastric adenocarcinoma with submucosal invasion, SMC was found in 100 (19.8%). Of the 27 GIST cases (median age, 60 years; range, 31-85 years; male/female ratio, 14:13), only 1 demonstrated SMC (4%), and the difference was significant (P = .0374). The clinicopathologic findings for cancer cases with SMC and without SMC are summarized in Table 1. The cases of cancer of the stomach with SMC were more frequently found in males, and there was a tendency for location in the upper part of the stomach; (2) only in the middle part of the stomach; (3) only in the lower part of the stomach; or (4) spread over 2 or more regions (Figure 1). There was no overall tendency regarding SMCs. Consequently, 31 cases with SMC and 33 cases without SMC were randomly selected and further analyzed for evaluation of gastritis and lifestyle habits.

Evaluation of Gastritis According to the Updated Sydney System

Figure 2 shows the results of the evaluation of gastritis. Intestinal metaplasia in the B2 region was more apparent in the background gastric mucosa with SMC than without SMC and in GIST. There was a tendency for higher scores for neutrophil infiltration at A1, B1, and B2; for H pylori at B2; for atrophy at A1, B1, and B2; and for intestinal metaplasia at A1 and B1 in background gastric mucosa with SMC, although differences did not reach statistical significance. In comparison with GIST cases, the background gastric mucosa with and without SMC showed higher grades of atrophy and intestinal metaplasia at A1, B1, and B2. Furthermore, the background gastric mucosa with SMC showed a higher grade of neutrophil infiltration in A1 and B2 than in GIST.

Thickness of the Muscularis Mucosae

Figure 3 shows the comparison of the thickness of the gastric muscularis mucosae. The muscularis mucosae in the B1 region of the cases of cancer of the stomach with SMC (mean ± SD, 210 ± 87 µm) was significantly thicker than in the cases without SMC (mean ± SD, 167 ± 73 µm; P = .0490) and in the cases of GIST (mean ± SD, 142 ± 73 µm; P = .0030). The A1 and B2 regions in cases with SMC also showed thickening of the muscularis mucosae compared with the cases without SMC, but the difference was not significant.

Habits and BMI

Figure 4 shows results for the Brinkman index, alcohol consumption, and BMI. The Brinkman index for cases of cancer with SMC was significantly higher than that for cases without SMC (P = .0259). Furthermore, the indices for cases of cancer with and without SMC were significantly higher than those for GIST cases (P = .0002 and P = .0338, respectively). Alcohol consumption was also significantly higher in cases of cancer with and without SMC than in GIST cases (P = .0188 and P = .0212, respectively). The BMI values showed no differences.

Discussion

In this study, SMC was identified in 100 of 504 cases of cancer of the stomach. Yamagiwa et al reported the incidence of diffuse SMC (including single and multiple lesions) to be
Representative photographs of gastric submucosal cysts (SMCs). A, A semimacroscopic photograph of a case of gastric carcinoma with SMCs (H&E, ×2). *The area of cancer spread in the lamina propria. Higher magnification is shown in C (†) and D (‡). B, In the lamina propria, well-differentiated adenocarcinoma is identified (H&E, ×100). C, Dilated SMCs can be identified beneath the cancer (H&E, ×40). D, Cancer epithelium has spread into the cyst wall. Cancer epithelium and nonneoplastic epithelium are identified in a dilated gland (H&E, ×200).
10.7% (160 of 1,500 resected stomachs), but carcinomas were present only in 253 of the 1,500 cases. In our study using 504 cancer cases, the incidence of SMC with gastric carcinoma was 19.8%, which was higher than their rate, probably because we examined cancer cases only. In GIST cases, SMC was found in only 1 (4%) of 27, underlining a difference in the status of the background mucosa from that of gastric carcinoma cases.

Clinicopathologic features of gastric carcinoma with SMC were proven to be characteristic in the present study,

Figure 2: Comparison of gastritis grades. A1, lesser curvature of the antrum; B1, lesser curvature of the body; and B2, greater curvature of the body. * P < .05. † P < .01. ‡ P < .001. GIST, gastrointestinal stromal tumor; SMC, submucosal cyst.
with links to male sex and location in the upper gastric portion. In the literature, esophagocardiac gastric cancers show a high male/female ratio (6.4:1), but that for cancers of the stomach with SMC was much higher, at 15.7:1, in our study. It has been reported that 69.8% of early cancers in the upper third of the stomach are of the differentiated type, and in our cases with SMC, the percentage was 80.0%.

The SMC is considered to be a result of repeated erosion and regeneration of the mucosa due to gastritis, causing epithelial components to enter into the submucosal layer, although definite evidence is lacking. Therefore, we examined the degree of gastritis using the Updated Sydney System. Intestinal metaplasia in the B2 region was significantly more severe in the background gastric mucosa with than without SMC. In addition, the background mucosa of GIST showed lower grades of atrophy and intestinal metaplasia than cases with SMC. In line with our results, it is reported that the grade of intestinal metaplasia is severe in gastric carcinoma associated with SMC, although the data were obtained without comparison with cancer without SMC.

It is also considered that intestinal metaplasia develops and spreads in association with repeated defects and repair of the mucosa. Furthermore, intestinal metaplasia is thought to be a precancerous lesion, particularly for differentiated adenocarcinoma. Therefore, it is compatible that the cancer lesions with background SMC tended to be of the differentiated type.

Although significant differences were not found, the degrees of *H pylori* infection, neutrophil infiltration, chronic inflammation, and glandular atrophy were also relatively high in stomachs with SMC. SMC may be a postinflammatory change following infection by *H pylori*, which may result in a high incidence of gastric cancer development. The lack of a significant difference for *H pylori* infection might be due to the disappearance of *H pylori* after the development of metaplastic mucosa.
In addition to gastritis, we searched for other factors associated with SMC development. Thickness of the muscularis mucosae and a smoking habit showed clear differences. Little attention has been hitherto given to the thickness of muscularis mucosae in cancers of the stomach with SMC. With inflammatory bowel diseases such as ulcerative colitis, the muscularis mucosae is thickened in correlation with disease duration, suggesting tissue remodeling due to chronic inflammation in a recent study by Mitsushashi et al. It is established that a smoking habit and alcohol consumption are related to cancer development in the oral cavity, esophagus, and gastric cardia. As for alcohol consumption, our results showed significantly higher alcohol consumption in cancer cases than in GIST cases. However, in GIST cases, the male/female ratio was 14:13, which is different from the ratios of cancer cases. This difference may influence the results. In fact, when we compared the groups consisting of only male cases, the difference was not observed (data not shown). On the other hand, the Brinkman Index for cases of cancer with SMC was also significantly higher than for cases of cancer without SMC (P = .0321) and GIST cases, when focusing on male cases (P = .0199). Therefore, it may be considered that smoking also influences damage to the gastric epithelium with gastritis and, thus, facilitates SMC formation.

Gastric cancers found in the background mucosa with SMC showed characteristic clinicopathologic features. SMC formation may be caused by gastritis and influenced by smoking.

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