Acta Med. Nagasaki 48 : 129－133

Circulating Sialyl Lewis^a, Sialyl Lewis^x, and Sialyl Tn Antigens in Patients with Diffuse Type of Gastric Cancer

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The aim of this study was to clarify whether or not preoperative serum levels of sialyl Lewis^a (CA19-9), sialyl Lewis^x (SLX), and sialyl Tn (STN) antigens are predictors for diffuse type gastric cancer. Eighty-two patients with diffuse type and 96 patients with intestinal type cancers were studied. Univariate logistic regression analysis showed that the following factors were significantly associated with diffuse type cancer: high levels of serum STN, young age (<62 years), female gender, tumor in the middle stomach, macroscopic type 3/type 4 cancer, presence of lymphatic invasion, peritoneal dissemination, stage III/IV, and non-curative resection. Multivariate analysis revealed that diffuse type cancer was independently related to young age (<62 years), female gender, tumor in the middle stomach, and macroscopic type 3/type 4 cancer. In conclusion, none of the preoperative serum levels of CA19-9, SLX, and STN were predictors for diffuse type cancer.

ACTA MEDICA NAGASAKIENSIA 48 : 129－133, 2003

Key Words: *sialyl Lewis^a; sialyl Lewis^x; sialyl Tn; CEA; gastric cancer; diffuse type; intestinal type.*

Introduction

Gastric cancer has been divided histologically into an intestinal type and a diffuse type[1,2]. There has been a dramatic and unexplained decrease in the incidence of gastric cancer, and this has occurred primarily in the intestinal type. However, unless gastric cancer is identified early, the prognosis for cure remains poor[1]. The diffuse type gastric cancer has been associated with a poorer prognosis than the intestinal type[1－4]. Therefore, preoperative prediction of diffuse type gastric cancer is important to establish strategies for perioperative adjuvant chemotherapy.

Sialyl Lewis^a (CA19-9), sialyl Lewis^x (SLX), and sialyl Tn antigen (STN) antigens are among the mucin-associated carbohydrate moieties found in large quantities in gastric tumors[5]. CA19-9 and SLX represent examples of type 1 and type 2 terminal carbohydrate structures, respectively. STN is an example of a carbohydrate core structure[5]. These three antigens may play an important role in cell adhesion. Their key role in local tumor invasiveness and metastasis has been previously discussed[6,7]. These carbohydrate antigens have also been detected in the sera of gastric cancer patients[8,9]. Thus far, a correlation has been reported between serum levels of carbohydrate antigens and diffuse or intestinal type gastric cancer[9－12]. However, in these reports, clinical and pathological data were evaluated only by univariate analysis. We believe that the results of univariate analysis should be confirmed by multivariate analysis.

In this study, we examined the preoperative serum levels of CA19-9, SLX, and STN in gastric cancer patients who underwent gastrectomy. The aim of this study was to clarify whether or not the serum levels of these carbohydrate antigens are predictors for diffuse type gastric cancer. We also evaluated the serum CEA levels in the same patients with gastric cancer because CEA is commonly used to evaluate recurrence and prognosis of gastric cancer patients after surgery[1].

Patients and Methods

Patients

A total of 178 gastric cancer patients (120 males and 58 females) were included in this study. The median age of patients was 62.0 years (range, 29 to 86

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years). All patients underwent resection for a gastric tumor at Nagasaki University Hospital and Sasebo Municipal Hospital between 1991 and 1993. Patients who had a synchronous or metachronous cancer of the stomach were excluded from this study. None of the patients had any evidence of other organ malignancies or history of preoperative treatment with anticancer drugs.

The following standardized procedures were performed: (i) gastric resection (the gastric resection line was 3 cm from the macroscopic edge for localized tumors and 5 cm for infiltrating tumors); (ii) prophylactic lymph node dissection greater than removal of group 1 lymph nodes[13]; and (iii) complete excision of invaded organs, irrespective of the number of sites on the organs, provided that there was no evidence of incurable factors such as peritoneal dissemination, liver metastasis, and widespread nodal involvement[14]. One hundred-twenty three patients underwent partial gastrectomy, and 55 patients had a total gastrectomy. The number of patients who underwent curative resection was 153. Twenty-five patients had noncurative resection. None of the patients died within 30 postoperative days. Written informed consent was obtained from each patient. At the time these data were analyzed, the median follow-up was 61.5 months (range: 42 days to 154.6 months).

Intestinal and diffuse types gastric cancer

Pathologic diagnoses and classification of the resected gastric cancer tissues were made according to the Japanese Classification of Gastric Carcinoma[13]. The histological classification was determined based on the predominant pattern of tumor as follows: papillary adenocarcinoma, tubular adenocarcinoma, poorly differentiated adenocarcinoma, signet-ring cell carcinoma, and mucinous carcinoma. Papillary or tubular adenocarcinoma can be interpreted as intestinal type, whereas poorly differentiated adenocarcinoma or signet-ring cell carcinoma can be regarded as diffuse type. Mucinous carcinoma can be interpreted as either intestinal or diffuse type, depending upon other predominant elements (papillary adenocarcinoma, tubular adenocarcinoma, poorly differentiated adenocarcinoma, or signet-ring cell carcinoma)[13].

Measurement of the serum levels of antigens

To detect the presence of circulating cancer markers, venous blood was obtained from patients after an overnight fast. Blood samples were immediately separated by centrifugation and sera were stored at -80℃. Serum levels of CA19-9, SLX, STN, and CEA were measured by the Otsuka Assay Laboratory (Tokushima, Japan) using the following commercially available radioimmunoassay kits: the Centocor CA19-9 RIA kit (Centocor, Malvern, PA, USA)[15], the FH-6 "Otsuka" kit (Otsuka Assay Lab., Tokushima, Japan)[16], the STN "Otsuka" kit (Otsuka Assay Lab., Tokushima, Japan)[17], and the CEA Roche 2 kit (Nippon Roche K.K., Tokyo, Japan)[18], respectively. The data obtained were based on simultaneous assays for each antigen using the same set of sera. According to the manufacturers' instructions, the diagnostic cut-off values for each marker were 37 U/ml for CA19-9, 38 U/ml for SLX, 45 U/ml for STN, and 2.5 ng/ml for CEA[15-18]. We classified patients into two groups based on the serum levels of each antigen: a high antigen group, serum antigen concentration greater than the recommended cut-off value; and a low antigen group, concentrations less than the cut-off value.

Statistical analysis

Logistic regression analysis was chosen to study the predictive value of risk factors because the outcome variable was binary (intestinal type cancer versus diffuse type cancer)[19, 20]. Potentially predictive variables were identified using a significance level of $P<0.25$ by univariate analysis, and these variables were then used in multivariate analysis. A 25% significance level was selected based on the recommendations of Hosmer and Lemeshow for building multivariate models[20].

Two variables with continuous data, such as age and maximal tumor diameter, were classified in two groups based on the median values (62.0 years and 4.0 cm, respectively). The disease-specific interval was calculated according to the Kaplan-Meier method[21], and differences between disease-specific intervals were tested for significance using the log rank test[22].

All tests were two-tailed and a P value of less than 0.05 was considered significant.

Results

Of the 178 gastric cancers, 96 (53.9%) were classified as intestinal type cancers and 82 (46.1%) were classified as diffuse type cancers.

Comparison of disease-specific survival after gastrectomy between patients with intestinal type cancer and those with diffuse type cancer

The survival time of patients with diffuse type

cancer was significantly shorter than the survival time of patients with intestinal type cancer (P=0.035) (Fig. 1). The cumulative 5-year survival rates were as follows: 59.8% among patients with diffuse type cancer, and 74.2% among patients with intestinal type cancer.

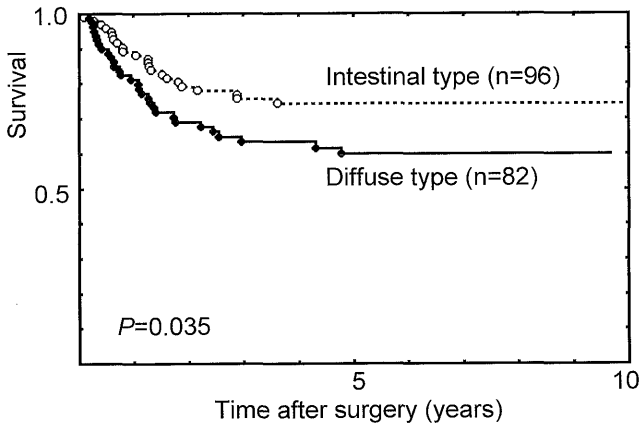

Figure 1. Comparison of disease-specific survival between patients with intestinal type cancer and those with diffuse type cancers of the stomach.

Logistic regression analysis to determine the predictive factors for diffuse type cancer

As shown in Table 1, 18 variables were included in this analysis. Univariate analysis showed that the following ten variables were significantly associated with diffuse type cancer: age, gender, tumor location, macroscopic type, serosal invasion, lymphatic invasion, peritoneal dissemination, stage, operative curability, and serum STN status.

To avoid the problem of collinearity in multivariate analysis, the variables 'stage' and 'operative curability' were excluded. By means of univariate analysis, 11 variables (age, gender, maximum tumor diameter, tumor location, macroscopic type, serosal invasion, lymphatic invasion, venous invasion, lymph node metastasis, peritoneal dissemination, and serum STN status) were identified at a significance level of P<0.25 for diffuse type cancer. These variables were therefore included in the multivariate analysis. Consequently, the diffuse type cancer was found to be independently related to four variables: young age (<62 years), female gender, tumor located in the middle stomach, and macroscopic type 3 or type 4 cancer. Of note, serum STN status was not related with diffuse type gastric cancer.

Table 1. Logistic regression analysis of predictive variables for diffuse type gastric cancer.

	Univariate analysis		Multivariate analysis	
Variables	OR (95% CI)*	*P* value	OR (95% CI)*	*P* value
Age				
≥62 years (n=95)	1		1	
<62 years (n=83)	3.29 (1.77-6.11)	0.0002	3.83 (1.71-8.60)	0.0010
Gender				
Male (n=120)	1		1	
Female (n=58)	5.25 (2.62-10.51)	<0.0001	4.75 (2.05-11.02)	0.0003
Maximum tumor diameter				
<4.0 cm (n=87)	1		1	
≥4.0 cm (n=91)	1.74 (0.96-3.17)	0.068	1.56 (0.57-4.29)	0.38
Tumor location				
Lower stomach (n=63)	1		1	
Middle stomach (n=77)	2.40 (1.20-4.80)	0.013	3.15 (1.28-7.76)	0.012
Upper stomach (n=38)	2.00 (0.87-4.58)	0.099	2.13 (1.73-6.17)	0.16
Macroscopic type				
Type 0 (n=77)	1		1	
Type 1 or Type 2 (n=47)	1.20 (0.56-2.59)	0.64	2.52 (0.70-9.07)	0.15
Type 3 or Type 4 (n=54)	6.21 (2.86-13.49)	<0.0001	6.65 (1.46-30.30)	0.013
Serosal invasion				
Absent (n=127)	1		1	
Present (n=51)	2.59 (1.32-5.09)	0.0053	1.85 (0.61-5.56)	0.27
Lymphatic invasion				
Absent (n=74)	1		1	
Present (n=104)	2.15 (1.16-4.00)	0.014	0.85 (0.29-2.58)	0.78
Venous invasion				
Absent (n=120)	1		1	
Present (n=58)	1.72 (0.91-3.26)	0.092	0.72 (0.25-2.03)	0.53
Lymph node metastasis				
Absent (n=94)	1		1	
Present (n=84)	1.78 (0.98-3.24)	0.059	0.67 (0.23-1.90)	0.45
Peritoneal dissemination				
Absent (n=164)	1		1	
Present (n=14)	4.80 (1.28-18.03)	0.019	2.32 (0.38-14.10)	0.36
Liver metastasis				
Absent (n=166)	1			
Present (n=12)	0.82 (0.25-2.73)	0.75		
Stage				
I (n=92)	1			
II (n=21)	1.55 (0.59-4.06)	0.37		
III (n=35)	2.27 (1.02-5.05)	0.042		
IV (n=30)	2.56 (1.09-5.98)	0.029		
Operative procedures				
Partial gastrectomy (123)	1			
Total gastrectomy (n=55)	1.32 (0.70-2.51)	0.39		
Operative curability†				
Curative (n=153)	1			
Noncurative (n=25)	2.55 (1.37-4.75)	0.0030		

Serum CA19-9 status				
Low CA19-9 group (n=141)	1			
High CA19-9 group (n=37)	0.75 (0.36-1.58)	0.45		
Serum SLX status				
Low SLX group (n=147)	1			
High SLX group (n=31)	1.12 (0.51-2.44)	0.78		
Serum STN status				
Low STN group (n=150)	1		1	
High STN group (n=28)	2.42 (1.04-5.62)	0.039	1.25 (0.37-4.31)	0.72
Serum CEA status				
Low CEA group (n=146)	1			
High CEA group (n=32)	0.76 (0.35-1.67)	0.50		

* OR, odds ratio; CI, confidence interval.

† In accordance with the criteria of the Japanese research Society for gastric carcinoma[13].

Discussion

In order to improve upon clinicopathological correlation, Lauren divided gastric cancer into intestinal and diffuse types[1,2]. The intestinal type has distinct large glands that are usually lined by well-polarized columnar cells with a well-developed brushed border. In the diffuse type of gastric cancer, the glandular structure is rarely present, and cells are scattered either as solitary cells or small clusters of cells. Typically, the intestinal type is better circumscribed than the diffuse type[1,2,13]. The intestinal type gastric cancer appears to show a preference for liver metastasis, whereas the diffuse type is more likely to metastasize to the peritoneum and lymph nodes[1-4]. The diffuse type gastric cancer has been associated with a poorer prognosis than the intestinal type[1,4]. The current study also revealed concordant results; i.e., the survival time of patients with diffuse type cancer was significantly shorter than the survival time of patients with intestinal type cancer.

In order to establish the strategy of preoperative (neoadjuvant) and/or postoperative adjuvant chemotherapy, a useful tumor marker that is able to predict diffuse type gastric cancer would be necessary. Thus far, no correlation between serum levels of CA19-9 or SLX and the Lauren's classification been reported[9-11]. Our current results were concordant with the results of their previous study. The current study revealed no correlation between serum CEA levels and Lauren's classification. However, Kodera et al.[10] reported that high serum CEA levels had a tendency to be associated with the intestinal type of gastric cancer (P=0.085). Kodama et al.[11] also reported a significant association between high serum CEA levels and the intestinal type of cancer. However, this result was based on a small number of patients with gastric cancer (6 intestinal and 40 diffuse type cancers). Thus, although high serum levels of CEA appear to be associated with the intestinal type of gastric cancer, these studies were evaluated by univariate analysis. The results of univariate analysis should be confirmed by multivariate analysis in a large number of patients.

Takahashi et al.[12] reported no significant correlation between serum STN levels and the diffuse or intestinal type of gastric cancers. In the current study, the univariate analysis demonstrated that among the four carbohydrate antigens (CA19-9, SLX, STN, and CEA), only high serum levels of STN was significantly associated with diffuse type cancer. However, the multivariate analysis showed that the level of serum STN was not an independent predictor for diffuse type.

Some studies reported that immunohistochemical expression of STN did not significantly correlate with Lauren's classification (the intestinal or diffuse type cancer)[23-26]. However, some reports have revealed that the intestinal type of cancers express STN more often than diffuse type cancers.[27,28]. Thus, the correlation between STN expression and Lauren's classification is controversial. Ikeda et al.[29] reported that the spread of STN into the surrounding stroma in diffuse type cancer may be associated with peritoneal dissemination in Stage IV gastric cancer. We previously reported that preoperative serum levels of STN predict liver metastasis and poor prognosis in patients with gastric cancer. However, the high serum levels of STN was associated with the diffuse type cancer[30]. Based on these results, we speculate that STN may not correlate with Lauren's classification (the intestinal or diffuse type c ancer), even though STN has an important role in metastasis of gastric cancer.

Body fluid (particularly blood and urine) testing for molecular markers (biomarkers) is easily accessible and useful in patients. The prognostic significance of circulating DNA in plasma or serum, and its genetic alterations in cancer are important trends[31]. In the current study, among the four carbohydrate antigens (CA19-9, SLX, STN, and CEA), we could not find an independent marker that predicted for the diffuse type gastric cancer. Further study using biomarkers is needed to clarify this issue.

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