Clinical Significance of Serum Soluble Intercellular Adhesion Molecule 1 in Gastric Cancer

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ABSTRACT

We studied the correlation between serum soluble intercellular molecule 1 (sICAM-1) and clinicopathological features in patients with gastric cancer. The impact of sICAM-1 on prognosis was also evaluated. The sera from 224 patients with gastric cancer, 44 healthy individuals, and 35 patients with benign gastrointestinal diseases (4 patients with submucosal stomach tumors, 6 patients with gastric ulcers, 1 patient with Crohn disease, 2 patients with ulcerative colitis, 7 patients with gall stones, 5 patients with chronic pancreatitis, and 10 patients with liver cirrhosis) were measured for sICAM-1 titer using a sandwich enzyme immunoassay method. There was no correlation between the serum titer of sICAM-1 and the age or gender of healthy controls. Among patients with benign gastrointestinal diseases, the patients with liver cirrhosis had a significantly higher mean serum sICAM-1 titer than that of healthy controls ($P < 0.0001$). The mean serum sICAM-1 titer of all patients with gastric cancer was not significantly different from that of healthy controls. However, among the patients with stage IV and recurrent disease, the serum sICAM-1 titer of those with hematogenous metastasis was significantly higher than that of patients without hematogenous metastasis ($P = 0.001$). The patients with a high serum sICAM-1 titer of more than 304 ng/ml (mean of healthy controls plus SD) showed a significantly worse prognosis than patients with a low serum sICAM-1 titer ($P = 0.010$). Nevertheless, serum sICAM-1 titer was not an independent predictor of prognosis by multivariate analysis. In conclusion, serum sICAM-1 cannot be used as a tumor marker for early diagnosis. However, sICAM-1 in sera may still be worthwhile to measure for monitoring hematogenous metastasis.

INTRODUCTION

ICAM-1, a member of the immunoglobulin superfamily, is a $M_r$ 70,000–110,000 type I transmembrane glycoprotein with five immunoglobulin-like extracellular domains (1). ICAM-1 is a ligand for the β2 integrin LFA-1 (2). It has been reported that high expression of ICAM-1 on a tumor cell’s surface increases the susceptibility of such tumor cells to lymphocyte-mediated tumor cytototoxicity through the ICAM-1/LFA-1 system (3, 4).

Although the source of sICAM-1 has not been fully elucidated, it can be released by cancer cells (4, 5) as well as by peripheral blood mononuclear cells, endothelial cells, and fibroblast cells (6). Proteolytic cleavage of membrane-bound ICAM-1 may be the most likely mechanism for the generation of sICAM-1 (7). Another feasible mechanism for sICAM-1 production could be alternative splicing of ICAM-1 mRNA lacking the intramembrane and intracellular domains. However, the corresponding spliced mRNA has not been identified (7). It has been reported that in patients with ICAM-1-negative cancer, a high level of sICAM-1 may be shed from endothelial cells stimulated by interleukin 1α (8).

It is possible for sICAM-1 to bind to the LFA-1 molecules of leukocytes and inhibit the binding of cell surface ICAM-1 on cancer cells with leukocytes (9, 10). Thus, production of sICAM-1 has been thought to play a role in avoiding ICAM-1/LFA-1-mediated tumor cell cytototoxicity. In the present study, we analyzed the sera from 224 patients with gastric cancer to clarify the clinical significance of serum sICAM-1 levels in this disease.

PATIENTS AND METHODS

Patients. The subjects chosen for this study were 224 patients with histologically confirmed gastric cancer who were admitted to the First Department of Surgery, Osaka City University Hospital (Osaka, Japan). Serum from each of these patients was obtained on admission. The serum samples were stored at $-20^\circ$C until assayed. A total of 217 patients had primary disease, and 7 patients had recurrent cancer. The mean age ± SD of patients was 60.3 ± 11.8 years (range, 31–89 years). There were 157 males and 67 females. Patients who had other carcinomas besides gastric cancers were omitted from the study. Gastric cancer patients with inflammatory disease, autoimmune disease, or liver dysfunction were also excluded from this study because it has been reported that serum sICAM-1 levels are elevated in these diseases (11–13). Throughout this report, the Japanese Classification of Gastric Carcinoma (14) was used for the pathological diagnosis and classification of variables. The histological type was divided into two groups: (a)
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The clinical significance of sICAM-1 in gastric cancer was 231 ± 6 ng/ml. The titer was considered to be statistically significant.

In the study, serum samples from 44 healthy individuals (controls) and 35 patients without gastric cancer but with benign gastrointestinal diseases (4 patients with submucosal stomach tumors, 6 patients with gastric ulcers, 1 patient with Crohn disease, and 2 patients with ulcerative colitis, 7 patients with gallstones, 5 patients with chronic pancreatitis, and 10 patients with liver cirrhosis) were also measured.

**Assay.** The sera were assayed for sICAM-1 with a quantitative sandwich enzyme immunoassay using Parameter Human sICAM-1 immunoassay kits (R&D Systems, Inc., Minneapolis, MN) according to the manufacturer’s instructions. The antibodies in the kit were raised against recombinant sICAM-1 and are used to quantify recombinant and natural human sICAM-1 accurately. No cross-reactivity was found with human IgG, soluble vascular cell adhesion molecule 1, or soluble E-selectin.

In brief, 100 μl of appropriately diluted antibody to recombinant human sICAM-1 conjugated to horseradish peroxidase and 100 μl of a 1:21-diluted serum sample or the known concentration sICAM-1 control were pipetted into the wells of a microplate that had been precoated with a murine monoclonal antibody specific for human sICAM-1. After mixing, the plate was covered with plate sealer and incubated at room temperature for 1.5 h. Each well was aspirated and washed thoroughly six times with a buffer; 100 μl of substrate (tetramethylbenzidine) were added for color development. The mixture was incubated at room temperature for 30 min. The color reaction was terminated by the addition of 100 μl of stop solution (acid solution). The optical density of each well was determined with an E max microplate reader (Molecular Devices Co., Sunnyvale, CA) set to 450 nm; wave correction was set to 630 nm. Each sample and the sICAM-1 control were examined twice. The concentration of each serum sample was determined by calculating the concentration of sICAM-1 corresponding to the mean absorbance from the standard curve using the sICAM-1 control.

**Statistical Analysis.** The correlation between the sICAM-1 titer and age was assessed by linear regression using the least-squares method. We used the unpaired t test to compare the sICAM-1 titer between the two groups. The χ² test was used to compare the prevalence or distribution of two variables. The relationship between the sICAM-1 titer and survival was examined by constructing Kaplan-Meier survival curves and by analyzing the differences using the log-rank test. The Cox proportional hazards model was used for the multivariate analysis of survival. P values less than 0.05 were considered to be statistically significant.

**RESULTS**

**Serum sICAM-1 Titer in Healthy Controls.** The mean ± SD of the serum sICAM-1 titer in 44 healthy controls was 231 ± 73 ng/ml. There was no correlation between serum sICAM-1 titer and age as examined by linear regression analysis ($r = 0.123; P = 0.425$). No significant difference was observed in serum sICAM-1 titer between 23 healthy males and 21 healthy females (mean ± SD, 236 ± 71 versus 226 ± 76 ng/ml; $P = 0.627$).

**Serum sICAM-1 Titer in Patients with Benign Gastrointestinal Diseases.** The serum sICAM-1 levels of patients with benign gastric disease, inflammatory bowel disease, gall stones, or chronic pancreatitis were not significantly different than those of healthy controls. However, patients with liver cirrhosis had significantly higher serum sICAM-1 titers than healthy individuals ($P < 0.0001$; Fig. 1).

**Serum sICAM-1 Titer in Patients with Gastric Cancer.** There was no statistically significant difference between serum sICAM-1 titers in patients with gastric cancer (mean ± SD, 222 ± 119 ng/ml) and in healthy controls ($P = 0.637$). However, some patients with advanced gastric cancer showed very high serum sICAM-1 titers (Fig. 2). The two patients with stage IV disease who had extremely elevated levels of serum sICAM-1 (997 and 994 ng/ml) had hematogenous metastases. These patients died from cachexia very shortly after admission; the patient with bone metastasis had a survival of 1.5 months, and the other patient with multiple liver metastases had a survival of 2.5 months. Patients with stage IV and recurrent disease were divided into two groups according to the status of hematogenous metastasis; this included metastases to the liver, bone, and skin. The serum sICAM-1 titers of those with hematogenous metastasis were significantly higher than the titers of those without hematogenous metastasis (mean ± SD, 414 ± 256 versus 222 ± 81 ng/ml; $P = 0.001$; Fig. 3).

The correlations between serum sICAM-1 titers and clinicopathological features are given in Table 1. Information about the histological type of all specimens was available because all 224 patients underwent endoscopic biopsy or surgery. Peritoneal or hepatic metastasis status was determined by imaging study and/or intraoperative findings. A total of 203 patients had sero-
sal invasion, as determined by intraoperative findings and/or histological examination. A total of 192 patients were diagnosed by histological examination with lymph node metastasis, and 188 patients were diagnosed with lymphatic and venous invasion by histology. There was no significant difference between the serum sICAM-1 titers of those with differentiated cancers and those with undifferentiated cancers or between those with and without peritoneal metastasis, serosal invasion, or venous invasion. There were significant differences in the serum sICAM-1 titers between those with and without hepatic metastasis (P = 0.0001), lymph node metastasis (P = 0.003), and lymphatic invasion (P = 0.049).

Impact of Serum sICAM-1 Titer on Survival in Gastric Cancer. All patients with gastric cancer were divided into high and low titer groups using a reference serum value of a sICAM-1 concentration of 304 ng/ml, which was the mean plus 1 SD of the serum sICAM-1 concentration observed in healthy control subjects. The survival rate of the high titer sICAM-1 group was significantly poorer than that of the low titer sICAM-1 group.

Fig. 2 The distribution of serum sICAM-1 titer in each stage of gastric cancer (stage I–IV), as defined by the Japanese Classification of Gastric Carcinoma, and in recurrent disease (R). The numbers in parentheses refer to the number of cases examined. Some advanced cases had very high serum sICAM-1 titers, although there was no significant difference between the titers of all patients with gastric cancer and healthy individuals.

Fig. 3 The distribution of serum sICAM-1 titer in patients with stage IV and recurrent disease with and without hematogenous metastasis. ○, no hematogenous metastasis; ●, liver metastasis positive; ■, bone metastasis positive; ▲, skin metastasis positive. The serum sICAM-1 titer in patients with hematogenous metastasis was significantly higher than that in patients without hematogenous metastasis, as shown by unpaired t test (P = 0.001).

Table 1 Correlation between clinicopathological features and serum sICAM-1 titer

<table>
<thead>
<tr>
<th>Feature</th>
<th>No. of cases</th>
<th>sICAM-1 (ng/ml)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Histological type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Differentiated</td>
<td>121</td>
<td>218 ± 118</td>
<td>0.880</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>103</td>
<td>220 ± 119</td>
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<tr>
<td>Peritoneal metastasis</td>
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<td></td>
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<tr>
<td>Negative</td>
<td>192</td>
<td>211 ± 107</td>
<td>0.099</td>
</tr>
<tr>
<td>Positive</td>
<td>32</td>
<td>245 ± 100</td>
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</tr>
<tr>
<td>Hepatic metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>210</td>
<td>207 ± 85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>372 ± 227</td>
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<tr>
<td>Serosal invasion</td>
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<td></td>
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</tr>
<tr>
<td>Negative</td>
<td>137</td>
<td>205 ± 79</td>
<td>0.323</td>
</tr>
<tr>
<td>Positive</td>
<td>66</td>
<td>218 ± 111</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>127</td>
<td>195 ± 83</td>
<td>0.003</td>
</tr>
<tr>
<td>Positive</td>
<td>65</td>
<td>235 ± 102</td>
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<tr>
<td>Lymphatic invasion</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>105</td>
<td>197 ± 79</td>
<td>0.049</td>
</tr>
<tr>
<td>Positive</td>
<td>83</td>
<td>224 ± 107</td>
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<tr>
<td>Venous invasion</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>137</td>
<td>201 ± 87</td>
<td>0.052</td>
</tr>
<tr>
<td>Positive</td>
<td>51</td>
<td>231 ± 106</td>
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</table>

*Values are expressed as mean ± SD.

Ps were determined by unpaired t test.

Fig. 4 Probability of survival for all gastric cancer patients studied in relation to their serum sICAM-1 titers. The reference value between high and low serum sICAM-1 concentration was defined as 304 ng/ml, which represents the mean plus 1 SD of the serum sICAM-1 concentration observed in healthy control subjects. The survival rate of the high titer sICAM-1 group was significantly poorer than that of the low titer sICAM-1 group.
Clinical Significance of sICAM-1 in Gastric Cancer

In patients with certain malignancies, the serum sICAM-1 titers have been found to be elevated in association with tumor growth and distant metastasis of malignant melanoma (15), lung cancer (16), breast cancer (17), hepatocellular cancer (11), and colorectal cancer (18). Poor survival of cancer patients correlated with a high level of serum sICAM-1 has also demonstrated (15, 17).

Several studies have emerged describing serum sICAM-1 in patients with gastric cancer. Benekli et al. (19) reported that serum sICAM-1 levels were significantly increased in 27 patients with gastric cancer compared to 18 healthy controls. Furthermore, sICAM-1 levels had no significant impact on survival, according to univariate analysis. Patients examined in the Benekli et al. study (19) had, for the most part, very advanced gastric cancer: 21 patients had stage IV cancer; and 19 patients had distant metastases. Kaihara et al. (9) showed that the serum level of sICAM-1 was significantly elevated in 30 patients with early gastric cancer and 30 patients with advanced disease was compared to 11 healthy controls. Velikova et al. (20) reported that serum sICAM-1 levels were significantly elevated in both 15 patients with stage I/II disease and 30 patients with stage III/IV disease as compared with 52 healthy subjects. However, Velikova et al. (20) found no statistically significant difference between the survival periods with high and low sICAM-1 levels when they used a cut-off value of the 95th percentile in healthy controls. Yoo et al. (21) studied sera from 142 patients with gastric cancer. With regard to inoperable cancer, the sICAM-1 level of 12 patients with liver metastasis was significantly greater than that of 35 patients without liver metastasis. Synchronous sICAM-1 and soluble vascular cell adhesion molecule 1 was an independent risk factor in gastric cancer patients of the Yoo et al. study (21). These previous investigations of serum sICAM-1 levels in gastric cancer consisted of small numbers of patients, and the majority of the patients had advanced disease. Therefore, the association between serum sICAM-1 levels and clinicopathological factors has not been fully elucidated. We studied the serum sICAM-1 titer of 224 patients with gastric cancer, including 122 stage I cases, and examined details of clinical significance regarding this molecule in the sera. Contrary to previous reports, there was no elevation of serum sICAM-1 titer in patients with gastric cancer in comparison with healthy controls. The discrepancy between the present results and previous reports may be caused by a substantial difference in the number of patients with early-stage disease. In our study, some patients with advanced gastric cancer had higher serum sICAM-1 titers. We also investigated the possible influences of hematogenous status on the elevation of serum sICAM-1 levels in patients with stage IV and recurrent disease. Among patients with these very advanced diseases, patients with hematogenous metastasis had higher serum sICAM-1 titers than those without hematogenous metastasis. In all patients studied, we found no significant influences on serum sICAM-1 titer according to histological type, peritoneal metastasis status, or serosal invasion status. However, metastasis in the liver or lymph nodes was associated with a rise in the serum sICAM-1 level. These results suggest that an increase in the bulk of a metastatic tumor might raise the sICAM-1 serum level because peritoneal metastasis usually does not create large tumors, whereas hepatic and lymph node metastasis do create large tumors in some cases.

The mechanism by which human cells generate sICAM-1 is not known. However, it has been demonstrated that sICAM-1 may be produced by the proteolytic cleavage of membrane-associated ICAM-1; whereas it may lack the intracellular and transmembrane region required for cell anchorage, sICAM-1 may possess most of the necessary extracellular structure to retain the functional activities of ICAM-1 (4, 7). It has been reported that ICAM-1 on the surface of cancer cells or antigen-presenting cells (i.e., macrophages) is a costimulatory factor that stabilizes T-cell receptor-mediated binding between these cells and T lymphocytes (22). sICAM-1 would work as an immunosuppressive agent by blocking LFA-1 on T lymphocytes, thus rendering it less available for binding with cell surface ICAM-1 on cancer cells (10). In this manner, the shedding of sICAM-1 may enhance the metastatic process by escaping host immune surveillance. This therefore presents an additional potential mechanism for high serum levels of sICAM-1 in patients with gastric cancer that had metastasized via hematogenous and lymphatic routes.

In conclusion, there was a modest association between serum sICAM-1 titer and cancer staging. The serum sICAM-1 level cannot be considered as an independent prognostic factor in patients with gastric cancer, although it can be a prognostic predictor by univariate analysis. Together with these results, serum sICAM-1 has limited clinical utility as a tumor marker; however, it may be useful for monitoring hematogenous metastasis. Measuring the serum sICAM-1 level might let us learn how sICAM-1 helps cancer cells evade immune surveillance.

REFERENCES


