Serum YKL-40 as a marker for cervical adenocarcinoma

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Background: The current study examined the clinical usefulness of YKL-40 in detection and prognosis of uterine cervical cancer.

Patients and methods: Serum levels of YKL-40, cancer antigen 125 (CA 125), carbohydrate antigen 19-9 (CA19-9), and squamous cell carcinoma (SCC) antigen were determined by enzyme-linked immunosorbent assay in women with benign gynecologic disease (n = 24), cervical malignancy (SCC, n = 104; adenocarcinoma, n = 37), and age-matched healthy controls (n = 45). Immunohistochemical analysis for local YKL-40 expression was carried out on 28 adenocarcinomas.

Results: Receiver operating characteristic curve analysis showed that YKL-40 [area under the curve (AUC) = 0.882] was significantly better at discriminating adenocarcinoma from healthy control than SCC antigen, CA 125, and CA19-9. For SCC, YKL-40 (AUC = 0.898) carried out similarly to SCC antigen and was better than CA 125 and CA19-9. Using a cut-off YKL-40 value of 92.2 ng/ml, sensitivity of YKL-40 in stage I adenocarcinoma (68%) was higher than that of the other three markers (11%–21%). Tumor-associated macrophages showed immunoactivity for YKL-40 in 2 of 28 adenocarcinoma tissue samples, but adenocarcinoma cells themselves were nonimmunoreactive in all samples. Multivariate Cox regression analysis revealed that elevated pretreatment YKL-40 levels predicted unfavorable prognosis, independent of International Federation of Gynecology and Obstetrics stage and age at diagnosis.

Conclusions: Pretreatment serum YKL-40 level is a possible prognosticator of cervical adenocarcinoma.

Keywords: adenocarcinoma, cervical, tumor marker, YKL-40

introduction

The incidence of adenocarcinoma of the uterine cervix has recently been increasing relative to that of squamous cell carcinoma (SCC) of the cervix [1]. Several serum markers such as cancer antigen 125 (CA 125), carbohydrate antigen 19-9 (CA19-9), and carcinoembryonic antigen have been clinically used in the management of cervical adenocarcinoma. These markers are not, however, as useful as serum SCC antigen is in the management of cervical adenocarcinoma. These markers are not, however, as useful as serum SCC antigen is in the management of cervical adenocarcinoma of the cervix, in terms of both specificity and sensitivity [2–5]. Development of new tools that support and facilitate the detection and management of adenocarcinoma is needed.

YKL-40, also known as CHI3L1 or human cartilage glycoprotein-39, is a mammalian chitinase-like protein, a group of proteins that act as growth factors for several cell types without exhibiting chitinase activity. YKL-40 has been suggested to play a pathophysiological role in tissue remodeling and inflammation, but its true function remains to be determined. Recent studies have revealed that YKL-40 has elevated serum levels in several solid tumors and that it is a potential biomarker in the detection and management of adenocarcinoma of several organs, including the colon, ovary, lung, and breast [6–10].

In the present study, we aimed to determine whether serum YKL-40 level was a useful biomarker for cervical adenocarcinoma. We compared pretreatment serum levels of YKL-40 in patients with cervical SCC, adenocarcinoma, or benign gynecologic disease and in healthy controls in order to assess the sensitivity of YKL-40 as a detection marker of cervical cancer. Moreover, in cervical adenocarcinoma, we also examined correlations with prognostic data to assess the role of YKL-40 as a prognostic marker. Pretreatment serum levels of YKL-40 were elevated in both adenocarcinoma and SCC of the cervix, even in the early stages. Pretreatment YKL-40 level was a predictor of disease-free survival (DFS) for adenocarcinoma, independent of International Federation of Gynecology and Obstetrics (FIGO) stage.

patients and methods

patients

A series of 141 patients with histologically confirmed cervical cancer (SCC, n = 104; adenocarcinoma, n = 37) were enrolled in this study.
Adenosquamous carcinoma was classified as adenocarcinoma. Of these patients, who were hospitalized at Chiba University Hospital from December 1999 to March 2004, 65 underwent primary surgery and the remaining 76 were given primary concurrent chemoradiation. The healthy control group consisted of 45 healthy age-matched female volunteers without cancer or joint, liver, metabolic, or endocrine disease. The benign control group consisted of 24 age-matched women without known cancer but with benign gynecologic disease: uterine fibroids (n = 6), endometriosis (n = 5), mature cystic teratoma (n = 5), and benign epithelial ovarian tumor (n = 8).

Patients were staged according to the FIGO guidelines. Lymph node metastases were diagnosed either by lymphadenectomy or by contrast-enhanced computed tomography detection of nodes with diameters >1 cm. Tumor size was measured by magnetic resonance imaging, and the greatest dimension was recorded. Informed consent to participate in the study was obtained from all patients before blood specimens were collected.

Characteristics of patients are listed in Table 1.

### serum assays

Blood samples were collected from patients before surgery or chemoradiation and sera were stored at −80°C until analysis. Serum YKL-40 concentrations were determined with a commercial ELISA kit (Quidel Corporation, Santa Clara, CA) according to the manufacturer’s recommendations. The detection limit of the YKL-40 assay was 20.0 ng/ml. Assays for SCC antigen, CA 125, CA19-9, and C-reactive protein (CRP) were carried out at the clinical chemistry laboratory of Chiba University Hospital. Upper limits of normal according to the manufacturers were 1.5 ng/ml for SCC antigen, 35 U/ml for CA 125, 37 U/ml for CA19-9, and 0.2 ng/ml for CRP.

### immunohistochemistry

Three micrometer-thick sections were stained with rabbit anti-human YKL-40 polyclonal antibody (Quidel Corporation) using an ABC kit (Vector Laboratories, Burlingame, CA). The sections were microwaved quickly for antigen retrieval and incubated at 4°C overnight with the YKL-40 antibody at a dilution of 1:400. Sections of glioblastoma were used as positive controls, and fibroblasts and blood vessels appeared in the cervical cancer sections as internal negative controls.

### statistical analysis

Serum YKL-40 levels showed a skewed distribution (Kolmogorov–Smirnov test). Statistical analysis was thus carried out on the log-transformed data, which were normally distributed. Values were recomputed and presented as geometric means or medians [11]. A 95% confidence interval (CI) for the ratio of the geometric means between two groups was calculated [12]. The transformed values were compared between two groups using the unpaired t-test or among three or more groups using one-way analysis of variance. Statistical analysis comparing area under the receiver operating characteristic (ROC) curve [area under the curve (AUC)] was carried out using the method of DeLong et al. [13]. Independent prognostic factors were determined by multivariate Cox regression analysis using a forward stepwise selection procedure. Age, lymph node status, tumor size, FIGO stage, YKL-40 levels, and CA125 levels were included in the analysis. DFS was estimated using the Kaplan–Meier method. Relationships between clinicopathological variables and DFS were assessed with the log-rank test. Statistical analysis was carried out using SPSS 15 and STATA/SE 8 software.

### results

#### distribution of YKL-40 levels and determination of cut-off value

Logarithms of YKL-40 levels in each group were normally distributed. The geometric mean and median YKL-40 levels for the normal control groups were 50 ng/ml and 56 ng/ml, respectively (range 20–127 ng/ml). These values are consistent with those given in the manufacturer’s instructions (Metra Biosystems). The 90th percentile of YKL-40 values for the normal control group, 92.2 ng/ml, was used as the cut-off value for the following analyses, with the exception of the survival analysis as explained later.

#### pretreatment YKL-40 levels were elevated in women with cervical malignancy

Pretreatment YKL-40 levels were higher in women with cervical malignancy than in women without it (Figure 1). However, pretreatment YKL-40 levels did not differ significantly between SCC and adenocarcinoma or between the two control groups.

Sensitivity and specificity of YKL-40 and other markers are summarized in Table 2. Pretreatment YKL-40 was above the cut-off level in 75% (78 of 104) of patients with SCC and 78% (29 of 37) of those with adenocarcinoma, whereas it was as low as 25% (6 of 24) in the benign control group (P < 0.001). Sensitivity of YKL-40 (75%) was comparable to that of SCC.
YKL-40 was the best biomarker of uterine cervical cancer

The utility of pretreatment YKL-40 level in detection of cervical cancer was evaluated using ROC analysis. As shown in Figure 2A, in terms of discrimination of SCC patients from normal controls, YKL-40 had a similar ROC curve to that of SCC antigen and demonstrated significantly superior performance to CA19-9 and CA 125. In contrast, YKL-40 was significantly better than all the other markers in distinguishing adenocarcinoma patients from normal controls (Figure 2B). The ROC analysis revealed that a clear cut-off value for which the ability to distinguish adenocarcinoma patients from normal controls (89.2 ng/ml) was maximized was close to the

antigen in SCC (72%) and was considerably greater ($P < 0.001$ for all) than that of the other markers for either of two histological types of cervical cancer.
cut-off initially determined on the basis of the 90th percentile in normal controls (92.2 ng/ml). With this cut-off level, the sensitivity and specificity of YKL-40 for adenocarcinoma were 78% and 89%, respectively.

As shown in Figure 3, YKL-40 level increased with advancing clinical stage \( (P < 0.0001) \). We then evaluated the performance of YKL-40 in terms of detection of early-stage cancer (Table 2). The sensitivity of YKL-40 for adenocarcinoma was as high as 68% and 71% in stages I and II, respectively; this was significantly higher \( (P < 0.001 \text{ for all}) \) than the sensitivities of the other three markers for stages I and II adenocarcinoma. For stage I SCC, the sensitivity of YKL-40 (60%) was again higher than that of SCC antigen (42%), but this difference did not reach statistical significance \( (P = 0.17) \).

**relationship between pretreatment YKL-40 level and clinicopathological variables in adenocarcinoma**

The relationships between YKL-40 levels and various variables are summarized in Table 3. At the time of this analysis, 28 patients were in tumor-free remission, five had persistent disease, and four had disease recurrence. Pretreatment YKL-40 levels showed a significant correlation with FIGO stage and with relapse or persistent disease status, while correlations with nodal status and tumor size were marginal.

**pretreatment YKL-40 level was a prognostic factor**

To evaluate the prognostic impact of YKL-40 levels, the association of pretreatment YKL-40 levels and survival outcome (DFS) was examined with Cox regression analysis (Table 4). A second cut-off level for YKL-40 level predicting DFS was determined to be <130 ng/ml, on the basis of the results of a ROC analysis of disease-free status versus pretreatment YKL-40 level (data not shown). For CA 125, a cut-off level of 30 ng/ml was used for prediction of survival as previously reported \[4\].

Among the six parameters examined, univariate analysis revealed that YKL-40 level \( (\geq 130 \text{ ng/ml}) \), age and FIGO stage (III + IV) were significantly associated with risk of relapse or persistence of adenocarcinoma. YKL-40 level \( \geq 130 \text{ ng/ml} \) increased the risk of relapse or persistent disease by 17 times. In multivariate Cox regression analysis, YKL-40 level \( \geq 130 \text{ ng/ml} \), FIGO stage (III + IV), and age at diagnosis were associated with relapse or persistent disease. YKL-40 level \( \geq 130 \text{ ng/ml} \) was a predictor of poor survival, independent of FIGO stage.

Kaplan–Meier analysis also revealed that patients with an elevation of serum YKL-40 \( (\geq 130 \text{ ng/ml}) \) were significantly more likely to have recurrence and persistent of disease \( (P < 0.0001) \) (Figure 4).

**relationship between YKL-40 and CRP levels**

YKL-40 increases in inflammatory conditions \[14\] and CRP are elevated in advanced cervical cancer because of local...
immunohistochemistry was also negative for adenocarcinoma cells in all cases examined.

**discussion**

In the present study, we demonstrated that serum YKL-40 level was elevated in the two major histological types of cervical cancer: SCC and adenocarcinoma. This nonpreferential elevation seems to be unique to YKL-40 because the other serum markers of cervical cancer, SCC antigen and CA 125, are rather specific for SCC and adenocarcinoma, respectively. It is also consistent with previous findings that serum YKL-40 level is increased in several solid tumors with a variety of histological types [16]. Like CRP, YKL-40 can be produced in the liver in response to bacterial lipopolysaccarides and we did detect a significant, albeit weak, positive correlation between serum YKL-40 and CRP levels in this study. However, serum YKL-40 appears to be more than a nonspecific biomarker of inflammation because it was superior to CRP in discriminating cervical cancer patients from tumor–free controls.

To date, no good indicator has been available for cervical adenocarcinoma that is comparable to SCC antigen for SCC. CA 125 has been considered the best marker for cervical adenocarcinoma among the established markers, but its sensitivity is 34% at best [2–4]. The sensitivity of YKL-40 for adenocarcinoma shown in the present study (78% for all disease and 68% for stage I tumors) was clearly higher than that of CA 125. ROC and AUC analysis also revealed that YKL-40 had significantly greater ability to distinguish patients with adenocarcinoma from healthy control individuals than did the other markers. Hence, YKL-40 seems to be the best biomarker to detect cervical adenocarcinoma. However, it is still not good enough for screening because sensitivity is inadequate (78%), even at the cut-off level of 92.2 ng/ml that gives modest specificity (90%).

Our study showed that preoperative YKL-40 level correlated with relapse or persistent disease and was a negative prognostic variable, independent of clinical stage and age of patients with cervical adenocarcinoma. In contrast, CA125, recognized as another prognostic indicator of the cervical adenocarcinoma [3, 4], was no longer a significant prognostic variable when YKL-40 level was included in the multivariate analysis.

**Table 4.** Cox regression analysis for hazard of relapse or persistent disease in patients with cervical adenocarcinoma

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Hazard ratio</th>
<th>95% CI</th>
<th>P</th>
<th>Multivariate Hazard ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td>1.1</td>
<td>1.02–1.13</td>
<td>0.004</td>
<td>1.1</td>
<td>1.01–1.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Lymph node status b positive (versus negative)</td>
<td>2.6</td>
<td>0.76–8.62</td>
<td>0.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor size &gt;4 cm (versus ≤4 cm)</td>
<td>3.6</td>
<td>0.93–13.8</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIGO stage III + IV (versus I + II)</td>
<td>9.2</td>
<td>2.32–35.7</td>
<td>0.002</td>
<td>10</td>
<td>2.03–50</td>
<td>0.05</td>
</tr>
<tr>
<td>YKL-40 ≥130 ng/ml (versus &lt;130 ng/ml)</td>
<td>17</td>
<td>2.11–133</td>
<td>0.008</td>
<td>11</td>
<td>1.29–97</td>
<td>0.03</td>
</tr>
<tr>
<td>CA 125 ≥30 ng/ml (versus &lt;30 ng/ml)</td>
<td>2.0</td>
<td>0.6–6.5</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aStepwise likelihood ratio method.

*CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; CA 125, cancer antigen 125.
Similarly, lymph node metastasis, another recognized prognosticator, also became insignificant when YKL-40 levels were included in the analysis. Thus, pretreatment YKL-40 level may be a superior prognostic marker to CA 125 and nodal status in cervical adenocarcinoma. Pretreatment YKL-40 level has been postulated as an independent prognostic indicator of short recurrence-free interval and short overall survival for cancers of various organs [7–10, 17–20]. Monitoring YKL-40 levels during therapy and follow-up has also been suggested to be useful in breast and colon cancer. However, a larger study with a longer follow-up period is needed to determine whether YKL-40 has the same utility in cervical cancer.

Within malignant tissues, YKL-40 can be secreted from fully activated macrophages associated with the tumor and can be produced by tumor cells themselves or by nonmalignant cells, such as activated neutrophils and fibroblasts, chondrocytes, and synovial cells [21–23]. Tumor cells express and secrete YKL-40 in breast and colon cancer [16], while tumor-associated macrophages but not tumor cells express YKL-40 in lung cancer [24]. To determine the source of serum YKL-40 in cervical adenocarcinoma, we examined the local expression of YKL-40 and found that tumor cells were negative in all cases and that tumor-associated macrophages were positive in only 2 of 28 tumors. There are several possible explanations for this lack of immunoreactivity. First, the present immunohistochemical did not have sufficient sensitivity to detect faint signals. Secondly, macrophages or neutrophils that had been recruited to and activated in the cancer tissues may return to the circulation and express YKL-40 systemically, albeit at a low level. Thirdly, YKL–40 was produced by some distant organ in response to cervical tumor as CRP is produced in response to cytokines secreted from the tumor. Further study using comprehensive methods including immunohistochemistry with different antibodies and in situ hybridization is necessary to more accurately determine immunoreactivity for YKL-40.

In conclusion, pretreatment YKL-40 level was the most sensitive serum marker for early-stage cervical cancer of both adenocarcinoma and SCC types, but accuracy was insufficient for screening purposes. However, the present study suggested that pretreatment YKL-40 level was a potential predictor of DFS, independent of FIGO stage. Further studies are needed to examine the clinical usefulness of this marker for monitoring disease status.

**funding**

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**references**


