Preoperative Cervical Cytology and E-Cadherin Expression in Endometrial Cancer


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Abstract

Objective: It is reported that cervical cytology is a significant factor related to stage, tumor grade, nodal metastasis, recurrence and survival rate in endometrial cancer. Moreover, reduced expression of the cell adhesion molecule E-cadherin is associated with higher tumor grade and metastasis in endometrial cancer. The objective of this study is to evaluate the relationship between the results of cytological assessment of glandular cells in cervical cytology before surgery, prognostic factors, and E-cadherin expression in endometrial cancer.

Methods: Between 2004 and 2011, 263 patients with endometrial cancer at all stages were treated with hysterectomy. We reviewed preoperative cervical smears and assigned each to one of three categories: (1) Negative, (2) Atypical glandular cells (AGC), and (3) Adenocarcinoma (AC). The relationship of these cytology, prognostic factors, and E-cadherin expression was evaluated.

Results: Statistical significance in overall survival was shown for preoperative cervical cytology, age, surgical stage, histological type, tumor grade, myometrial invasion, cervical involvement, lymph node metastasis except peritoneal cytology. Patients with AC cytology were more likely than those with normal cervical cytology to have a higher International Federation of Gynecology and Obstetrics (FIGO) stage, poorer histopathology, higher tumor grade, deeper myometrial invasion, higher incidence of cervical involvement, and higher prevalence of lymph node metastasis. In addition, AC and AGC cytology appeared to be associated with a poorer prognosis and to have lower E-cadherin expression than negative cytology.

Conclusions: Cervical cytology may be a guide to prognosis in endometrial cancer, and E-cadherin expression may correlate with appearances of abnormal endometrial cells.

Keywords: Cytology; E-cadherin; Endometrial cancer; Prognosis

Introduction

Endometrial cancer is the most frequently diagnosed gynecological malignancy in the United States, with an estimated 43,470 new cases diagnosed in 2010 [1]. In Japan, endometrial cancer is currently the fourth most common gynecologic malignancy, with an estimated incidence of 6,665 new cases in 2010 [2]. Notably, however, the Japan Society of Obstetrics and Gynecology (JSOG) reported that endometrial cancer increased from 976 cases in 1983 to 4267 in 2005 and 6113 in 2009, and accounted for about half of all cases of malignant uterine disease [3]. The estimated 5-year overall survival for early-stage endometrial carcinoma is 82% but decreases remarkably to 67% for regional disease and 16% for distant disease. Although the majority of patients (approximately 83%) are diagnosed as having stage I or II disease, those with advanced-stage endometrial carcinoma have poor prognosis [4]. The treatment of endometrial cancer is primarily based on surgery, consisting of hysterectomy and bilateral salpingo-oophorectomy. There is no worldwide consensus whether pelvic and/or para-aortic lymphadenectomy should be performed as part of the staging procedure [5,6]. For endometrial cancer patients, the expensiveness of surgery depends on the presence of risk factors for metastatic disease, like high tumor grade, deep myometrial invasion, and cervical involvement [6]. However, preoperative assessment of these factors remains a challenge. Therefore, to predict prognosis in patients with endometrial cancer before surgery is important to evaluate the indications of therapies appropriately.

The Papanicolaou cervicovaginal test (Pap test) was designed to screen for squamous pathology of the cervix. In that regard, it has been a resounding success for decades. Often, moreover, atypical endometrial cells are also present incidentally on Pap tests. This provides cytopathologists with an opportunity to examine these cells in specimens obtained for other reasons, sometimes raising suspicion of significant abnormalities of the endometrium that otherwise may have gone undetected. Atypical glandular cells (AGC) represent a diagnostic category with features suggestive of adenocarcinoma but which are not sufficient for a definitive diagnosis in the Bethesda System (TBS) 2001 [7]. It has been reported that 3 - 32% of patients diagnosed with AGC have endometrial cancer, moreover the reported rates of AC cytology among patients with endometrial cancer range from 11% to 31% [8-11]. Pap test himself noted long ago that the vaginal smear method is not as accurate for diagnosing carcinoma of the fundus as it is for diagnosing carcinoma of the cervix [12]. Nevertheless, cervical cytology is a significant factor related to stage, tumor grade, nodal metastasis, recurrence and survival rate in endometrial cancer [10,11,13].

E-cadherin is one of the caretakers of the epithelial phenotype and is responsible for stable cell-cell contacts and adherens junctions. There is a direct correlation between lack of E-cadherin and loss of the epithelial phenotype [14]. Previous studies showed that reduced expression of

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the cell adhesion molecule E-cadherin is associated with higher tumor grade and metastasis in endometrial cancer [15].

The objective of this study is to evaluate the relationship between the results of cytological assessment of glandular cells in cervical cytology before surgery, prognostic factors, and E-cadherin expression in endometrial cancer.

Materials and Methods

Specimens

Between January 2004 and December 2011, 263 patients with endometrial carcinoma at all stages were treated with hysterectomy in Sapporo Medical University. None of these patients had received any form of tumor-specific therapy before surgical excision. Before surgical treatment, cervical specimens were collected from all patients, and cervical cytology was examined. We reviewed these cervical smears and assigned each to one of three categories: (1) Negative, (2) Atypical glandular cells (AGC), and (3) Adenocarcinoma (AC).

Immunohistochemistry

The cells collected from cervix were fixed with cold acetone on the slides. The fixed tissue cells were pre-incubated with a blocking solution (PBS containing 5% skimmed milk) for 30 min at room temperature. The sections were incubated with anti-E-cadherin antibody (Clone No., HECD-1; Takara) diluted 1:500 for 2 h, and washed in PBS. FITC-conjugated anti-mouse immunoglobulin diluted 1:200 in PBS was then added (Dakopatts, Copenhagen, Denmark) and the slides were incubated for 1 h. After incubation with secondary antibodies, the slides were washed in PBS, mounted in fluorescent mounting medium (Dakopatts) and examined by immunofluorescent microscopy (Nikon, Tokyo).

Tissue samples

One hundred eighteen specimens of these patients with endometrial adenocarcinoma were selected for immunohistochemistry. The specimens were formalin-fixed, paraffin-embedded and then cut into 1- to 2-µm-thick sections for H&E and immunohistochemical staining.

Immunohistochemistry

Tissue sections for immunohistochemistry were deparaffinized in xylene for 10 minutes, fixed in 100% ethanol for 5 minutes, and then rehydrated through a graded ethanol series. Thereafter, heat-induced epitope antigen retrieval was accomplished by immersing the sections in Target Retrieval Solution (Dako) and heating them at 100°C for 20 minutes at room temperature. After incubation with secondary antibodies, the slides were washed in PBS, mounted in immunofluorescent microscope (Nikon, Tokyo).

Results

The mean age of the patients was 59.5 years, with a range of 30 to 87 years. Examination of the cervical cytology revealed that of the 263 patients with endometrial carcinoma, 136 (51.7%) were Negative, 64 (24.3%) had AGC, and 63 (24.0%) had AC; 188 cases (71.5%) were surgical stage I, 28 (10.6%) were stage II, 37 (14.1%) were stage III, and 10 (3.8%) were stage IV. Histologic subtypes included 245 (93.2%) endometrioid adenocarcinomas (108 International Federation of Gynecology and Obstetrics (FIGO) grade 1, 93 grade 2 and 44 grade 3), 9 serous adenocarcinomas, and 9 others; 177 patients (67.3%) had less than 50% myometrial invasion, 86 (32.7%) had greater than 50%, 45 (17.1%) had cervical involvement, and 16 (6.1%) had positive peritoneal cytology. Pelvic or para-aortic lymphadenectomy was performed on all stage I-III patients and on 4 of the 10 stage IV patients. 27 (10.5%) of the 257 patients receiving lymphadenectomy showed pelvic or para-aortic lymph node metastasis. Univariate analysis of the value of various clinicopathological factors including preoperative cervical cytology in relation to overall survival is shown in Table 1. Table 1 contains exact formula: intensity score × proportion (0.0-1.0). The intensity score was defined as follows: negative staining (0), faint staining (1), moderate staining (2), and strong staining (3). In brief, the calculation formula is (0 × proportion + 1 × proportion + 2 × proportion + 3 × proportion), and the total score ranges from 0 to 3.0.

Statistical analysis

The clinical characteristics of the study population were summarized using descriptive statistics. Correlations between the cervical cytology, patient age, histologic parameters and E-cadherin staining were assessed using the t test or z test. Multivariate logistic regression analysis was performed to determine clinical factors associated with AC. Survival rates were estimated by Kaplan-Meier analysis. The log-rank test was used to compare survival curves. All statistical tests were performed using SPSS version 20.0 software (Tokyo, Japan), and 2-tailed p values <0.05 were considered statistically significant.
Figure 1 shows Kaplan-Meier analysis of overall survival for patients with each cervical cytology (Negative, AGC, or AC). Overall survival was significantly longer in Negative than AC (p < 0.001) and AGC (p = 0.033) using Log-rank test.

Table 2 summarizes the results of the preoperative cervical cytology (Negative, AGC, and AC) and clinicopathologic findings. No significant correlation was found between the patients' age and Pap smear findings. Of the 216 patients with FIGO stage I or II, 120 (55.6%) had Negative cytology and 40 (18.5%) had AC, whereas among the 47 patients with FIGO stage III or IV, 16 (34.0%) were Negative and 23 (48.9%) had AC (p < 0.001). Of the 245 patients with endometrioid type disease, 132 (53.9%) were Negative and 53 (21.6%) had AC, whereas among the 18 patients with non-endometrioid type disease, 4 (22.2%) were Negative and 10 (55.6%) had AC (p = 0.003). Of the 201 patients with Grade 1 or 2 endometrioid type, 45 (22.4%) had AGC and 39 (19.4%) had AC, whereas among the 44 patients with Grade 3, 15 (34.1%) had AGC (p = 0.016) and 14 (31.8%) had AC (p = 0.011). Of the 177 patients with less than 50% myometrial invasion, 101 (57.1%) were Negative and 33 (18.6%) had AC, whereas among the 86 patients with deeper myometrial invasion, 35 (40.7%) were Negative and 30 (34.9%) had AC (p = 0.004). Of the 218 patients without cervical involvement, 123 (56.4%) were Negative and 41 (18.8%) had AC, whereas among the 45 patients with cervical involvement, 13 (28.8%) were Negative and 22 (48.9%) had AC (p < 0.001). Of the 230 patients without lymph node metastasis, 125 (54.3%) were Negative and 47 (20.4%) had AC, whereas among the 27 patients with metastasis, 8 (29.6%) were Negative and 15 (55.6%) had AC (p = 0.001). No significant association was found between the cervical cytological findings and the patients' age or peritoneal cytological findings. On multivariate analysis, the only significant variable associated with AC were stage (OR = 4.496, 95%CI: 2.012-10.049) and cervical involvement (OR = 4.148, 95%CI: 1.839-9.353). There was no association with age, histological type grade, myometrial invasion, lymph node metastasis except peritoneal cytology in this study.

Figure 2: (A) Negative-Localization of E-cadherin is along plasma membrane. (B) AGC: E-cadherin appeared to be diffusely distributed along the cell-cell contacts. (C,D) AC: E-cadherin was diffused into cytoplasm and E-cadherin expression was decreased. (Representative images of immunostaining for E-cadherin (green); ×200)

values of Hazard ratio (HR) results from proportional hazard regression, together with exact values of designated probability and 95% confidence interval (CI). Statistical significance in overall survival was shown for preoperative cervical cytology, age, surgical stage, histological type, tumor grade, myometrial invasion, cervical involvement, lymph node metastasis except peritoneal cytology in this study.

Table 2: Relationship between preoperative cervical cytology and clinicopathologic findings.

Table 1: Univariate analysis of cervical cytology and prognostic factors in overall survival.

Table: Multivariate logistic regression analysis

Variables | OR (95% CI) | p value
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Stage (III, IV vs. I, II) | 4.496 (2.012-10.049) | <0.001
Cervical involvement | 4.148 (1.839-9.353) | <0.001

Figure 2 showed that Localization of E-cadherin is along plasma membrane in Negative (Figure 2A), however E-cadherin-specific fluorescence appeared to be diffusely distributed along the cell-cell contacts in AGC (Figure 2B), moreover E-cadherin was diffused into cytoplasm and E-cadherin expression was decreased in AC (Figures 2C and 2D).
Pathological features, cell morphology, protein expression, and genetic cytology appears to be associated with a poorer prognosis than negative cervical involvement, and higher prevalence of lymph node metastasis with AC were more likely than Negative to have poorer prognostic tumor grade [8,10,17,19], deeper myometrial tumor invasion [8,10], those with positive cervical cytology tend to be older [16] and have a higher risk of recurrence compared to patients with negative preoperative cervical cytology, which serve as a predictor of clinical parameters for endometrial cancer [11].

As compared to patients with negative preoperative cervical cytology, those with positive cervical cytology tend to be older [16] and have a higher FIGO stage [8,10,17,18], poorer histopathology [8,18], higher tumor grade [8,10,17,19], deeper myometrial tumor invasion [8,10], higher incidence of cervical involvement [8,18-20] and extraperitoneal spread, including positive peritoneal washing [10,21] and lymph node metastasis [8,18]. Our study similarly demonstrated that patients with AC were more likely than Negative to have poorer prognostic indicators, including a higher FIGO stage, poorer histopathology, higher tumor grade, deeper myometrial invasion, higher incidence of cervical involvement, and higher prevalence of lymph node metastasis except patient's age and positive peritoneal washing. In addition, AC cytology appears to be associated with a poorer prognosis than negative cytology (p < 0.001).

Prognosis in endometrial cancer is associated with clinical or pathological features, cell morphology, protein expression, and genetic alterations. For endometrial cancer, inactivating mutations in PTEN and activating mutations in KRAS and PIK3CA have been reported to occur in 30-50, 10-30, and 30-40% of endometrial cancers, respectively [22]. Furthermore, mutations or over expressions of genes involved in these pathways have been associated with invasion metastasis, and prognosis of a variety of cancers, including endometrial cancer [23]. The PI3K/PTEN/AKT/mTOR pathway further interacts with the estrogen receptor at multiple levels, supporting potential crosstalk between estrogens and the PI3K pathway [24,25].

We previously reported that HEC-1A cells (moderately differentiated endometrioid cancer cells) show weaker cell adhesion and are more invasive than Ishikawa cells (well-differentiated endometrioid cancer cells) in 3D co-cultures of endometrial cancer cells and fibroblasts with human collagen sponges [29]. Moreover, our previous study showed that decreased expression of E-cadherin in endometrioid adenocarcinoma was associated with tumor dedifferentiation and myometrial invasion, and hypermethylation in the promoter region of the E-cadherin gene was correlated with tumor progression, tumor dedifferentiation, and the depth of myometrial invasion [30]. Cellular changes resulting in a more mesenchymal-like state driven by a pathological epithelial-mesenchymal transition (EMT) program in tumors are thought to play a significant role in carcinoma progression and are associated with a poor prognosis. The central target of EMT signaling pathways is repression of a variety of cancers, including endometrial cancer [23].

In summary, we found that

1. Patients with AGC or AC cytology was significantly poorer prognosis than Negative.
2. AC cytology is associated with several prognostic factors and AGC is associated with tumor grade.
3. E-cadherin expression is weaker in patients with AGC and AC cytology than Negative. We reported the association between cervical cytology and E-cadherin expression, however these results may indicate only small proportion because E-cadherin concerns various parts in endometrial cancer.

Table 3: Relationship between preoperative cervical cytology and E-cadherin expression.

<table>
<thead>
<tr>
<th>Cervical cytology</th>
<th>Expression score</th>
<th>Negative (N=53)</th>
<th>AGC (N=32)</th>
<th>AC (N=29)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-Cadherin</td>
<td>0.00 - 0.19</td>
<td>15</td>
<td>9</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.20 - 0.99</td>
<td>12</td>
<td>17</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.00 - 3.00</td>
<td>26</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.926</td>
<td>0.594</td>
<td>0.497</td>
<td>0.008†</td>
<td>0.015‡</td>
</tr>
</tbody>
</table>

T-test was performed. †: Negative vs AC. ‡: Negative vs AGC-EM

**References**


