endometrioid adenocarcinoma (6 of 25 patients), squamous differentiated adenocarcinoma (1 of 3), papillary serous (2 of 2) and undifferentiated cancer (1 of 1). The median time for the onset of effect was 2.0 months (range, 0.7–4.5) and the median duration of response was 1.8 months (range, 0.9–4.6). The median follow-up time was 17.6 months (range, 1.7–36.3) and median TTP was 3.9 months (95% CI, 1.5–10.2 months) (Figure 1). Median survival time was 17.8 months (95% CI, 7.4–22.0 months).

Safety and toxicity

In all, 33 patients were assessable for toxicity (Table 3). Also, 31 (94%) patients experienced grade 3 or 4 neutropenia, and three (9%) developed febrile neutropenia. Nonhaematologic toxicities included grade 3 anorexia and vomiting experienced by some patients (18 and 9%, respectively). One patient experienced grade 3 peripheral neuropathy (sensory and motor) after five treatment cycles. Three patients terminated the study as a consequence of the following toxicities: infection with *Mycobacterium avium* complex (one), grade 4 hypersensitivity reaction despite premedication with dexamethasone (one) and grade 3 oedema with pleural effusion after six treatment cycles (one). All three patients recovered after receiving recommended medical treatment. There were no treatment-related deaths.

Table 1 Patient characteristics

Characteristic	No. of patients ($n=33$)
Age, years	
Median	59
Range	39–74
ECOG performance status	
0	23
1	9
2	1
Disease status	
Stage III, IV	9
Recurrent	24
Histology	
Endometrioid	26
Adenocarcinoma with squamous differentiated	3
Papillary serous	2
Adenocarcinoma, unspecified	1
Undifferentiated	1
Tumour grade	
1	11
2	11
3	6
Unknown	5
Prior treatment	
Surgery	29
Radiotherapy	9
Hormonal therapy	5
Prior chemotherapy	
None	19
Doxorubicin and platinum	9
Platinum alone	3
Others	2

ECOG = Eastern Cooperative Oncology Group.

DISCUSSION

At initial diagnosis, only a small percentage of endometrial cancer patients have recurrent or advanced disease with distant metastases, and therefore a multicentre trial is essential for the accrual of patients. This multicentre phase II trial, although relatively small in sample size, clearly demonstrated that docetaxel is active in the treatment of endometrial cancer. Toxicity was manageable and predominantly haematologic.

Taxanes have shown activity in this setting previously, with paclitaxel demonstrating overall response rates of 27–37% when used as a single agent in endometrial cancer (Ball *et al*, 1996; Lissoni *et al*, 1996; Lincoln *et al*, 2003). Combination chemotherapy with paclitaxel and carboplatin or cisplatin has resulted in response rates of 50–56% (Dimopoulos *et al*, 2000; Hoskins *et al*,

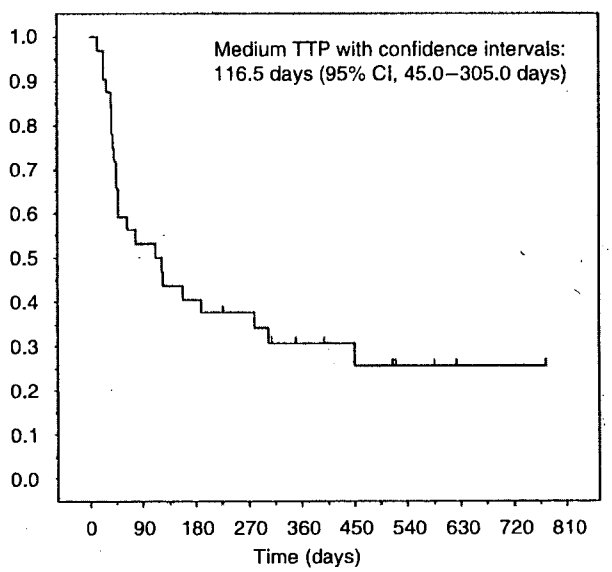

Figure 1 KM curve of estimated TTP.

Table 2 Best response (RECIST criteria) to docetaxel

	Prior chemotherapy ($n=13$)		No prior chemotherapy ($n=19$)		Total ($n=32$)	
Response	**No. of patients**	**%**	**No. of patients**	**%**	**No. of patients**	**%**
Complete response	0	0	1	5	1	3
Partial response	3	23	6	32	9	28
Stable disease	4	31	5	26	9	28
Progressive disease	5	38	6	32	11	34
Not assessable	1	8	1	5	2	6
ORR (95% CI)		23 (5.0–53.8)		37 (16.3–61.6)		31 (16.1–50.0)

ORR = overall response rate; CI = confidence interval.

Table 3 Adverse effects

	NCI-CTC grade ($n=33$)									
	1		2		3		4		3–4	
Toxicities	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Neutrophils	1	3	0	0	10	30	21	64	31	94
Haemoglobin	11	33	11	33	1	3	1	3	2	6
Lymphopenia	1	3	14	42	11	33	—	11	33	
Platelets	6	18	1	3	0	0	0	0	0	0
Alopecia	5	15	26	79	—	—	—			
Fatigue	13	39	7	21	3	9	0	0	3	9
Anorexia	12	36	5	15	6	18	0	0	6	18
Nausea	16	49	6	18	2	6	—	2	6	
Vomiting	7	21	3	9	3	9	0	0	3	9
Diarrhoea	14	42	3	9	3	9	0	0	3	9
Constipation	2	6	10	30	4	12	0	0	4	12
Stomatitis	3	9	5	15	1	3	0	0	1	3
Febrile neutropenia	—	—	3	9	0	0	3	9		
Infection	0	0	3	9	0	0	0	0	0	0
Oedema	7	21	3	9	1	3	0	0	1	3
Neuropathy-motor	1	3	0	0	1	3	0	0	1	3
Neuropathy-sensory	9	27	2	6	1	3	0	0	1	3
Supraventricular arrhythmia	0	0	0	0	1	3	0	0	1	3
Allergic reaction	3	9	0	0	0	0	1	3	1	3
Rash/desquamation	6	18	5	15	1	3	0	0	1	3
Injection site reaction	5	15	2	6	0	0	0	0	0	0
Nail changes	4	12	0	0	—	—	—			
AST	9	27	3	9	0	0	0	0	0	0
ALT	8	24	2	6	0	0	0	0	0	0
Hypokalaemia	0	0	—	3	9	0	0	3	9	

NCI-CTC = National Cancer Institute common toxicity criteria; AST = asparate aminotransferase; ALT = alanine aminotransferase. Present NCI-CTC grade 3–4 in >5% patients and breakdown if possible by whether patient had prior chemotherapy.

2001; Scudder *et al*, 2005). However, a GOG randomised trial of women with advanced or recurrent endometrial carcinoma, in which the combination paclitaxel–doxorubicin was compared with doxorubicin–cisplatin, showed that the paclitaxel arm did not result in an improved outcome (Fleming *et al*, 2000). A subsequent GOG study, in which the combination paclitaxel, doxorubicin and cisplatin (TAP) with G-CSF was compared with doxorubicin–cisplatin, showed that the TAP arm yielded a better response (57 *vs* 34%; $P<0.01$), progression-free survival (median, 8.3 *vs* 5.3 months; $P<0.01$) and OS (median, 15.3 *vs* 12.3 months; $P=0.037$) than the control arm. However, more grade 3 neuropathy (12 *vs* 1%) and congestive heart failure were observed with TAP than with doxorubicin–cisplatin (Fleming *et al*, 2004). In light of this imbalance between efficacy and toxicity, TAP has not been accepted as the standard chemotherapy regimen in routine clinical practice.

Docetaxel has a toxicity profile that is different from paclitaxel. In particular, neurotoxicity occurs at a low incidence with docetaxel. In our study, only one patient developed grade 3 neuropathy-sensory and recovered in several weeks. While fluid retention is a distinctive toxicity of docetaxel, this can be prevented using premedication (Piccart *et al*, 1997); in our trial, one patient developed pleural effusion since the routine premedication with corticosteroids was not applied.

Several studies have reported on second-line chemotherapy for endometrial cancer. Two phase II trials of second-line paclitaxel report response rates of 27% (12 out of 44) and 37% (7 out of 19) (Lissoni *et al*, 1996; Lincoln *et al*, 2003). An older report describes a 30% response rate to second-line high-dose cisplatin (3 mg kg^{-1}) among 13 patients (Deppe *et al*, 1980). With the exception of these studies, response rates to second-line chemotherapy are uniformly less than 20% and most are less than 10% (Slayton *et al*, 1982, 1988; Stehman *et al*, 1983; Thigpen *et al*, 1984b, 1986; Homesley *et al*, 1986; Asbury *et al*, 1990; Muss *et al*, 1991, 1993; Sutton *et al*, 1994; Rose *et al*, 1996; Muggia *et al*, 2002). In our study, 23% of pretreated patients (3 out of 13) had a PR to docetaxel, suggesting that it too is active as second-line therapy.

In conclusion, this multicentre phase II trial shows that docetaxel is active in the treatment of chemotherapy-naïve and chemotherapy pretreated patients with advanced or recurrent endometrial cancer and possesses a manageable toxicity profile; however, the effect was transient and accompanied by pronounced neutropenia in most patients. The exploration of the efficacy of docetaxel combinations in phase III studies for the treatment of endometrial cancer is of great interest and will be initiated.

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Appendix

The following institutions (with principal investigators) participated in this study: Sapporo Medical University, Sapporo, Satoru Sagae; Niigata University, Niigata, Kenichi Tanaka; Tochigi National Hospital, Utsunomiya, Masaaki Kikuchi; National hospital Organization Saitama Hospital, Wako, Mikio Mikami; National Cancer Center Hospital, Tokyo, Noriyuki Katsumata; Tokyo Women's Medical University, Tokyo, Hiroaki Ohta; School of medicine, Keio University, Tokyo, Daisuke Aoki; St. Marianna University School of Medicine, Kawasaki, Kazushige Kiguchi; Kitasato University, Sagamihara, Hiroyuki Kuramoto; Nagoya City University, Nagoya, Atsushi Arakawa; Fujita Health University, Toyoake, Yasuhiro Udagawa; Aichi Cancer Center Hospital, Nagoya, Kazuo Kuzuya; Osaka Medical College, Takatsuki, Ken Ueki; Kinki University, Osakasayama, Hiroshi Hoshiai; Hyogo Medical Center for Adults, Akashi, Ryuichiro Nishimura; Wakayama Medical University, Wakayama, Katsuji Kokawa; Okayama University, Okayama, Junichi Kodama; Okayama Red Cross General Hospital, Okayama, Kohei Ejiri; Kawasaki Medical School, Kurashiki, Ichiro Kohno; National Kyushu Cancer Center,

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Sensitivity to cisplatin determined by the histoculture drug response assay and clinical response of endometrial cancer

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Abstract. Kanasugi M, Aoki D, Suzuki N, Susumu N, Nakata S, Horiuchi M, Udagawa Y, Nozawa S. Sensitivity to cisplatin determined by the histoculture drug response assay and clinical response of endometrial cancer. *Int J Gynecol Cancer* 2006;16:409–415.

This study investigated the value of the *in vitro* histoculture drug response assay (HDRA) for predicting the efficacy of chemotherapy in patients with endometrial cancer. Specimens were obtained from 115 patients with endometrial cancer treated at Keio University Hospital between 1994 and 2002. Tumor fragments were cultured on collagen sponge gel with cisplatin for 7 days, and cell viability was assessed. The cutoff value of the 50% inhibitory concentration of cisplatin was set at 23 μg/mL. Sensitivity of stage III or IV disease to chemotherapy was investigated, and differences of 5-year progression-free survival between patients with sensitive and resistant tumors were evaluated by the Kaplan–Meier method. Tumors were evaluable in 93.0% of patients (107/115). Among 38 patients in stages III or IV, 23 received chemotherapy containing cisplatin. Seven sensitive tumors did not recur, while recurrence/progression occurred within 6 months in 8/16 patients with tumors showing low sensitivity. Among stages III and IV patients, there was a significant difference of 5-year progression-free survival ($P < 0.05$) between those with tumors showing high or low sensitivity. Accordingly, the HDRA may predict the efficacy of chemotherapy for endometrial cancer.

KEYWORDS: cisplatin, endometrial cancer, histoculture drug response assay.

Both irradiation and chemotherapy have become mainstream postoperative treatments for endometrial cancer and are often performed when patients have various clinicopathologic risk factors[1], although there is still some controversy as to which modality is more appropriate. At the 2003 Annual Meeting of the American Society of Clinical Oncology, the results of the GOG122 study were reported. This phase III randomized clinical study compared par-abdominal irradiation with doxorubicin plus cisplatin chemotherapy (AP therapy) in patients who had progressive endometrial cancer and demonstrated significantly longer progression-free survival and overall survival after AP therapy in comparison with radiotherapy. These findings suggested that chemotherapy could become the standard form of postoperative therapy for endometrial cancer[2].

Burke reported that the response rate to cyclophosphamide, doxorubicin, and cisplatin (CAP) therapy was 45% in 87 patients with progressive or recurrent endometrial cancer[3], while Thigpen *et al.* found a response rate of 45% to AP therapy[4]. At present, both CAP and AP therapy are widely used as first-line, multiple-agent adjuvant chemotherapy regimens for high-risk patients with endometrial cancer. However, many patients show an inadequate response to chemotherapy. Thus, a test that could assess the activity of anticancer agents against each patient's tumor might improve the response rate and would also help to avoid adverse reactions caused by the administration of ineffective drugs.

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Several *in vitro* drug sensitivity tests have been developed for solid tumors. In 1966, Kondo *et al.* reported the succinic dehydrogenase inhibitor method. In this method, a tumor cell suspension is mixed with an anticancer agent, and the viable cell count is determined by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide (MTT) assay[5,6]. Because the evaluation rate is relatively high (80%) and the true negative rate is 91–97%, this method is useful for excluding ineffective drugs and also has the advantage of being fairly rapid. However, contamination by fibroblasts can interfere with interpretation of the results, and the assay is not useful for assessing the sensitivity of time-dependent drugs because of difficulty in maintaining cultures for long enough. Furthermore, the true positive rate is only 60–70%, so the succinic dehydrogenase inhibitor method is not particularly helpful for selecting effective drugs.

In 1977, Hamburger and Salmon developed the human tumor clonogenic assay, which evaluates drug activity based on the number of colonies formed during culture on soft agar, and this method has been reported to be effective for predicting the tumor response[7]. Von Hoff *et al.* reported that the true negative and true positive rates of the assay were 91% and 69%, respectively[8], so it may be useful for excluding ineffective drugs as in the succinic dehydrogenase inhibitor method but would not be so useful for selecting effective drugs. Also, the tumor evaluation rate is only 30–70%, and the clonogenic assay has the disadvantages of being complicated and requiring 2–3 weeks for evaluation.

In 1989, Hoffman *et al.* developed the histoculture drug response assay (HDRA), in which tumor tissue blocks were cultured and sensitivity was evaluated from ^{3}H-thymidine uptake; they reported that evaluation was possible for more than 90% of tumors[9]. As a modification of this method, Furukawa *et al.*[10] transplanted various tumors (including stomach cancer, colon cancer, breast cancer, lung cancer, hepatocellular carcinoma, and neuroblastoma) into nude mice and assessed cell viability by the MTT assay. They obtained true positive, true negative, and accuracy rates of 89.8%, 90%, and 90%, respectively, and concluded that this method was useful for predicting the efficacy of anticancer agents[10]. We have also used the HDRA to assess the response of ovarian cancer to cisplatin, employing the MTT assay to determine cell viability because it is simpler than measuring ^{3}H-thymidine uptake. We achieved true positive, true negative, and accuracy rates of 88%, 86%, and 87%, respectively, and the tumor evaluation rate was also extremely high (97%), suggesting that the HDRA was a promising *in vitro* sensitivity test for predicting the response to chemotherapy[11].

With respect to the *in vitro* sensitivity testing of endometrial cancer, Ngyuyen reported on evaluation of cultured endometrial cells by the ATP chemosensitivity assay[12], while Hiramatsu *et al.* assessed cultured endometrial cancer cells by crystal violet staining[13]. However, both of these studies employed cultured tumor cells, and there have been no reports published regarding the relationship between *in vitro* sensitivity testing and the clinical response of endometrial cancer.

Accordingly, the present study was performed to assess the possible clinical application of the HDRA for predicting the efficacy of chemotherapy in endometrial cancer patients.

Materials and methods

Patients

The subjects of this study were 115 patients with endometrial cancer who underwent surgery between June 1994 and February 2002 at Keio University Hospital. Our institutional ethics committee approved the study protocol, and informed consent was obtained from all of the patients before the collection of tumor specimens. The FIGO surgical stage[14] was I, II, III, and IV in 64, 12, 29, and 10 patients, respectively. Among the 115 patients, 106 had endometrioid adenocarcinoma, which was well-differentiated adenocarcinoma (G1), moderately differentiated adenocarcinoma (G2), and poorly differentiated adenocarcinoma (G3) in 63, 29, and 14 patients, respectively. The other nine patients had serous adenocarcinoma ($n = 1$), clear-cell adenocarcinoma ($n = 4$), mucinous adenocarcinoma ($n = 2$), and carcinosarcoma ($n = 2$).

After surgery, 39 patients received chemotherapy, 16 patients received irradiation, and 60 patients had no further therapy.

Among the patients who received adjuvant chemotherapy containing cisplatin and underwent the HDRA, eight had measurable lesions (Table 1).

Methods

Collagen gel sponge (Gelfoam, Pharmacia & Upjohn, Kalamazoo, MI) was cut into approximately 1-cm^3 cubes and placed into the wells of a 24-well plate (Sumilon, Sumitomo Bakelite, Tokyo, Japan). As the culture medium, F-12 medium (Gibco Laboratories, Grand Island, NY) was prepared with 20% fetal bovine serum (Mitsubishi Chemical, Tokyo, Japan) and 80 μg/mL kanamycin (Meiji Seika, Tokyo, Japan),

Table 1. Cisplatin sensitivity in the HDRA and clinical response of patients with measurable lesions[a]

Case no.	IC_{50} (μg/mL)	Clinical response	Sites of measurable disease	Correlation	Chemotherapy
1	7.7	PR	Liver, parametrium	TP	CAP
2	16.9	CR	Lung	TP	CAP
3	21.5	PR	Liver	TP	CAP
4	23.4	PD	Local tumor, peritoneum	TN	CAP
5	33.4	PD	Peritoneum, lymph node	TN	CBDCA
6	34.7	NC	Parametrium	TN	TXL + CBDCA
7	115.0	PR	Lymph node	FP	CAP
8	151.8	PD	Lung	TN	CAP

[a]The response to treatment was classified as complete remission (CR), partial remission (PR), no change (NC), and progressive disease (PD). Correspondence with the HDRA was classified as true positive (TP), true negative (TN), or false positive (FP). The chemotherapy regimens included CAP (cyclophosphamide + doxorubicin + cisplatin), CBDCA (carboplatin), and TXL (paclitaxel) + CBDCA.

and 1 mL of this medium was added to each well. The concentration of cisplatin (Nippon Kayaku, Tokyo, Japan) in the medium was set at 6.25, 12.5, 25, 50, and 100 μg/mL, and four wells were used to test each concentration. Wells without cisplatin were also employed as a control.

Surgical specimens were collected aseptically and cut into approximately 1-mm^3 cubes with scissors. Then a tissue fragment was placed on the collagen gel in each well of the 24-well plate, and the specimens were incubated for 7 days at 37°C under an atmosphere of 5% CO_2. After incubation, the viable cell count was determined by the MTT assay. In brief, 100 μL of MTT (Sigma Chemical, St. Louis, MO) dissolved in phosphate buffered saline (5 mg/mL) containing 100 mM succinic acid (Wako Pure Chemical Industries, Osaka, Japan) was aseptically added dropwise to each well, and then incubation was continued for 4 h at 37°C under an atmosphere of 5% CO_2. Next, the tumor tissue specimens were transferred to a new 24-well plate, and MTT–formazan was extracted with dimethyl sulfoxide (1 mL/well) (Wako Pure Chemical Industries, Japan), after which 100 μL of the extract was transferred to each well of a 96-well microplate (Sumilon, Sumitomo Bakelite), and the absorbance at 540 nm was determined using a microplate reader (Model 450, Bio-rad Laboratories, Herculus, CA). The wet weight of each piece of tumor tissue was measured after extraction of MTT-formazan, and the absorbance per 1 g of tumor tissue was calculated. Then, the growth inhibition rate was calculated by the following equation, which compares the difference between treated and untreated cultures: Growth inhibition rate (%) = (1 − [absorbance per gram of tumor treated with cisplatin/absorbance per gram of tumor treated without cisplatin]) × 100.

A dose-response curve was plotted using the mean growth inhibition rate determined by testing four wells at each concentration, and the concentration that inhibited tumor growth by 50% (inhibitory concentration [IC_{50}] value) was calculated. Ideally, the cutoff IC_{50} value for evaluating sensitivity would be set by comparing the assay results with the actual clinical response, but there were only a few patients with measurable lesions in the present study. Therefore, the cumulative efficacy rate curve was plotted from the IC_{50} values obtained, and the cutoff IC_{50} value was set at 23 μg/mL based on the response rate (21%) of endometrial cancer to cisplatin monotherapy reported by Edmonson *et al.*[15] (Fig. 1). Tumor sensitivity was classified as high when the IC_{50} value was below the cutoff value, while it was classified as low when the IC_{50} value was above the cutoff value.

The response of the patients with measurable lesions was evaluated according to WHO criteria[16]

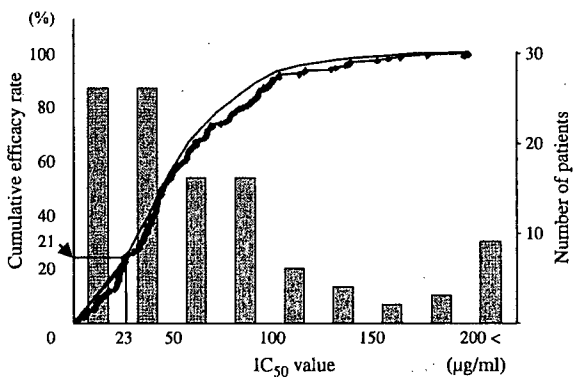

Figure 1. Cumulative efficacy of cisplatin-based therapy for endometrial cancer and histogram of the IC_{50} values of cisplatin. Because the clinical response rate of endometrial cancer to cisplatin alone was 21%, the intersection of this value with the cumulative efficacy curve was specified as the cutoff IC_{50} value for the HDRA (ie, 23 μg/mL). The HDRA was performed using specimens from 115 patients with endometrial cancer, and evaluation was possible in 106 patients. The mean IC_{50} value was 176.8 μg/mL.

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based on computed tomography of the thorax, abdomen, and pelvic cavity. To assess the relationship between the clinical response and tumor sensitivity in the HDRA, tumors showing complete remission or partial remission (PR) were classified as "responsive," while those showing no change or progressive disease were classified as "resistant."

Thirty-three of the 39 patients receiving chemotherapy had endometrioid adenocarcinoma. In these patients, Welch's *t* test was used to assess the differences in the sensitivity to cisplatin between G1, G2, and G3 disease.

If the tumor was found to have a high sensitivity by the HDRA and showed a response to chemotherapy, this was classified as a true positive result, while tumors judged to have a low sensitivity that showed resistance to chemotherapy were classified as true negative. Similarly, tumors with a high sensitivity in the HDRA that showed resistance to chemotherapy were classified as false positive, while lesions with a low HDRA sensitivity that responded to chemotherapy were classified as false negative. The accuracy rate was calculated from the following equation: Accuracy rate = (Number of true positive tumors + Number of true negative tumors)/Total number of evaluable tumors.

When patients receiving chemotherapy had measurable lesions, the response of these lesions was considered to directly reflect their sensitivity to drug therapy, and the tumor response was compared with the evaluation of sensitivity based on IC_{50} values. Since there were only a few patients with measurable lesions after surgery, sensitivity to chemotherapy was evaluated in most of the subjects by using recurrence/progression versus nonrecurrence as an indicator of efficacy. However, the prognosis of stage I or II endometrial cancer is relatively good[17] and the recurrence rate is very low, making it difficult to evaluate the relationship between tumor sensitivity and recurrence/progression. Therefore, this relationship was only assessed in patients with stage III or IV disease. The Kaplan–Meier method was used to investigate the significance of differences in 5-year progression-free survival between high and low sensitivity tumors, while the Cox–Mantel test was used to assess differences in the prognosis.

Results

Tumor evaluation rate

Evaluation was possible in 107 of the 115 patients tested by the HDRA, so the tumor evaluation rate was 93.0%. Evaluation was impossible because of poor color development during the MTT assay in seven patients and because of contamination in one patient.

Relationship between the HDRA and the clinical response

Figure 1 shows a histogram of the IC_{50} values. The mean IC_{50} value was 176.8 μg/mL. When the cutoff IC_{50} value was set at 23 μg/mL, 22 out of 106 evaluable patients (20.4% of all patients) were classified into the high-sensitivity group. Measurable lesions were present in 8 out of 115 patients (Table 1). Among them, three tumors showed a high sensitivity in the HDRA, and the clinical response was complete remission in one case and PR in two cases. On the other hand, five tumors were accessed to show a low sensitivity, with the actual response being PR, no change, and progressive disease in one, one, and three cases, respectively. Based on these results, the true positive rate, true negative rate, and accuracy rate were 100%, 80%, and 87.5%, respectively (Table 2).

Relationship between the HDRA and recurrence/progression of stage III or IV disease

Among the 115 patients, 38 were in stage III or IV, and 23 of them received chemotherapy containing cisplatin. Of them, 7 and 16 had tumors that showed high and low sensitivity in the HDRA, respectively. There was no recurrence of the highly sensitive tumors, while recurrence/progression occurred in 8 of the 16 patients with tumors showing a low sensitivity. Recurrence or progression occurred within 6 months after the start of treatment in seven of those eight patients (Table 3). When the clinical response to cisplatin and tumor sensitivity in the HDRA were compared based on the presence or absence of recurrence/progression, the true positive rate, true negative rate, and accuracy rate were 100%, 50%, and 65.2%, respectively (Table 4).

Table 2. Relationship between the HDRA and clinical response in patients with measurable lesions[a]

	Clinical response		
Sensitivity	Responsive	Resistant	Total
High	3	0	3
Low	1	4	5
Total	4	4	8

[a]The optimal cutoff value of cisplatin was set at 23 μg/mL based on the reported response rate. True positive rate = 3/3 = 100%, true negative rate = 4/5 = 80%, accuracy rate = 7/8 = 87.5%.

Table 3. Sensitivity according to the HRDA and recurrence/progression in stage III or IV patients

Case no.	Stage	IC_{50} (μg/mL)	Recurrence	Progression-free interval (months)	Chemotherapy	Observation period (months)
1	IIIA	1.5	–		CAP	20
2	IIIC	2.7	–		CAP	26
3	IVB	4.4	–		CAP	94
4	IVB	7.7	–		CAP	7
5	IVA	12.0	–		CAP	89
6	IIIC	14.0	–		CAP	73
7	IVA	21.5	–		CAP	5
8	IVB	23.4	+	2	CAP	7
9	IVA	23.6	–		CAP	14
10	IIIA	29.3	–		CAP	60
11	IIIC	33.4	+	5	CBDCA	5
12	IIIC	34.7	+	2	TXL + CBDCA	18
13	IIIA	35.0	–		CAP	9
14	IIIA	37.2	–		CAP	87
15	IIIC	45.6	–		CAP	8
16	IIIC	77.7	+	5	CAP	12
17	IIIC	105.8	–		CAP	17
18	IIIC	112.0	–		CAP	27
19	IIIC	115.0	+	2	CAP	4
20	IIIB	151.8	+	5	CAP	26
21	IIIC	200<	+	7	CAP	82
22	IVB	200<	–		CAP	14
23	IIIC	200<	+	4	CAP	5

CAP, cyclophosphamide + doxorubicin + cisplatin; CBDCA, carboplatin; TXL, paclitaxel + CBDCA.

Prognosis of stage III or IV disease

The 5-year progression-free survival rate was determined for 23 patients with stage III or IV disease. It was 100% for 7 patients with tumors that showed a high sensitivity in the HDRA and 42.9% for 16 patients whose tumors showed a low sensitivity, and there was a significant difference between these two groups ($P < 0.05$) (Fig. 2).

Sensitivity of endometrioid adenocarcinoma with differing histology

There were no significant differences in the cisplatin sensitivity of endometrioid adenocarcinoma between the different levels of histologic differentiation (Fig. 3).

Table 4. Relationship between sensitivity and recurrence/progression in stage III or IV patients[a]

	Recurrence/progression		
Sensitivity	Positive	Negative	Total
Positive	7	0	7
Negative	8	8	16
Total	15	8	23

[a]True positive rate = 7/7 = 100%, true negative rate = 8/16 = 50%, accuracy rate = 15/23 = 65.2%.

Discussion

In the present study, the tumor evaluation rate of endometrial cancer was 93% by the HDRA, which was clearly higher than with the succinic dehydrogenase inhibitor method and the human tumor clonogenic assay. This was similar to the evaluation rate for gastric cancer and large-bowel cancer (96.3%) reported by Furukawa *et al.*[18], and the rate for ovarian cancer (97%) reported by Ohie *et al.*[11]. Accordingly, our results suggest that the HDRA is also applicable to

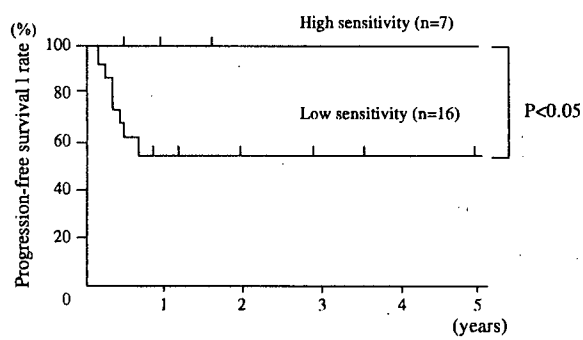

Figure 2. Prognosis of patients in stage III or IV. The 5-year progression-free survival rate of stage III or IV patients with highly sensitive tumors was 100%, while it was only 42.9% when the tumors were of low sensitivity, showing a significant difference between the two groups (Cox–Mantel test, $P < 0.05$).

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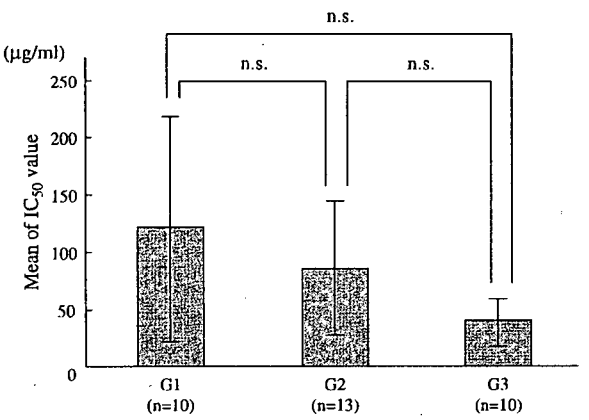

Figure 3. Cisplatin sensitivity of endometrioid adenocarcinoma with different grades of histologic differentiation. There was no significant difference of cisplatin sensitivity in the HDRA between different grades of endometrioid adenocarcinoma (G1, 10 patients; G2, 13 patients; and G3, 10 patients) (Welch's test).

endometrial cancer. The tumor evaluation rate may have been higher with the HDRA than the other methods because the original tissue architecture and tumor cell viability are maintained[18].

The cutoff IC_{50} value for the HDRA should ideally have been set by determining the value giving the maximum accuracy in comparison with the actual clinical response, but there were too few patients with measurable lesions in this series. Instead, a cumulative efficacy curve was plotted from the IC_{50} values obtained, and the cutoff value was based on the previously reported clinical response rate for cisplatin monotherapy (23 μg/mL). With this method, the accuracy rate of the HDRA was 87.5% in the eight patients who had measurable lesions. It fell to 75% when the cutoff IC_{50} value was set between 23 and 33 μg/mL or between 16 and 21 μg/mL. Accordingly, it seems appropriate to set the cutoff IC_{50} value at 23 μg/mL, but it will be necessary to confirm this in a larger number of patients with evaluable lesions. In ovarian cancer patients in whom the clinical response was evaluable, we previously investigated the extent of agreement between tumor sensitivity determined by the HDRA and the actual clinical response, and we confirmed that the cutoff value of the assay was appropriate because it was almost identical to that determined on the basis of the reported clinical response to cisplatin monotherapy[19]. Even with a cutoff IC_{50} value that was based on data reported previously, the accuracy rate of the HDRA was high in our patients with measurable lesions, as has been reported before[18,19].

Because the postoperative recurrence rate is very low in patients with stage I or II endometrial cancer[17], it was expected to be difficult to evaluate the efficacy of adjuvant therapy in such patients. Therefore, we evaluated the efficacy of chemotherapy based on recurrence/progression in patients with stage III or IV disease alone. Among them, we found that chemotherapy could prevent recurrence of advanced cancer, provided that the HDRA showed a high sensitivity. In the low-sensitivity group, recurrence tended to occur earlier, presumably because of the inadequate efficacy of chemotherapy. According to Blackledge[20], the response rate to repeat chemotherapy containing cisplatin was less than 10% among patients with ovarian cancer that recurred during initial cisplatin treatment or in whom the interval between initial treatment and recurrence was only 3–6 months. In the present study, recurrence occurred within 6 months of treatment in 87.5% (seven out of eight) of the patients from the low-sensitivity group with recurrence. These findings suggested tumor resistance to cisplatin, so the results of the HDRA may be related to the clinical response to chemotherapy. Because the prognosis of stage III or IV patients was better in the high-sensitivity group than the low-sensitivity group, it may be possible to improve survival by selection of cisplatin therapy based on the results of HDRA evaluation.

In the present study, there was no significant difference in cisplatin sensitivity between the different histologic grades of endometrioid adenocarcinoma, but the mean IC_{50} value decreased as the tumor grade became lower. This might have been due to more rapid growth of cancer cells in these lesions, reflecting the aggressiveness of low-grade tumors. To clarify this point, investigation of a larger number of tumors will be necessary.

Since the response rate to multiple-agent chemotherapy is usually only around 40–50%[21], this rate might be increased if the indications for chemotherapy could be determined by a drug sensitivity test that accurately predicted the effective agents. Selection of more effective drugs by sensitivity testing may also improve the quality of life by avoiding adverse reactions caused by ineffective agents.

No consensus has been reached as to whether chemotherapy or irradiation should be given postoperatively to patients with endometrial cancer, but the HDRA may help to determine which is more useful in the future, although it would be necessary to perform a randomized comparative clinical study. Since most of the patients in the present study received CAP therapy, the HDRA should possibly have been performed for all drugs in the chemotherapy regimen. Because of interactions among drugs, however, the sum of the sensitivities to the individual agents does not

necessarily reflect the efficacy of multiple-agent chemotherapy. Our finding that the sensitivity to cisplatin shown by the HDRA was correlated with the clinical response suggests that cisplatin is a key drug for endometrial cancer and that this test is useful to predict the prognosis when platinum-based therapy is given. Cisplatin is still widely used in many chemotherapy regimens for endometrial cancer, even though new drugs such as taxanes or topoisomerase inhibitors have been developed, so it remains a key drug in clinical practice. There was no significant difference in the cisplatin sensitivity of endometrioid adenocarcinoma with different levels of differentiation, presumably because of the relatively small number of tumors studied.

In conclusion, there was a strong correlation between sensitivity to cisplatin determined by the HDRA and the clinical response of endometrial cancer. Accordingly, this method may be useful for predicting the efficacy of chemotherapy and may provide useful information when selecting chemotherapy or radiotherapy as postoperative adjuvant therapy.

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Lactation and Risk of Endometrial Cancer in Japan: A Case-Control Study

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OKAMURA, C., TSUBONO, Y., ITO, K., NIIKURA, H., TAKANO, T., NAGASE, S., YOSHINAGA, K., TERADA, Y., MURAKAMI, T., SATO, S., AOKI, D., JOBO, T., OKAMURA, K. and YAEGASHI, N. *Lactation and Risk of Endometrial Cancer in Japan: A Case-Control Study.* Tohoku J. Exp. Med., 2006, **208** (2), 109-115 —— The incidence of endometrial cancer is rapidly increasing in Japan. Although the risk factors in European populations have been well described, there are few epidemiologic studies regarding risk factors for endometrial cancer in Japanese women. This hospital-based case-control study among Japanese women was carried out from 1998 to 2000. The cases were selected from women with endometrial cancer (n =155), and the controls selected from women attending the university gynecological outpatient clinic for cervical cancer screening (n = 96). Subjects were interviewed to ascertain breast feeding practices, contraceptive usage, as well as potential risk factors for endometrial cancer. We observed a lower risk of endometrial cancer associated with oral contraceptive (OC) and a higher risk associated with higher body mass index (BMI), and older ages at first and last delivery. Gravidity reduced odds ratio (OR) for endometerial cancer to 0.34 (95% confidence interval [CI] 0.13-0.92). Compared with parous women who had never breastfed, the multivariate OR for women with a history of breastfeeding was 0.37 (95% CI, 0.17-0.82). Additionally, a greater lapse of time since breastfeeding increased OR for endometrial cancer by over three times. In conclusion, the present study has indicated that breastfeeding reduces the risk of endometrial cancer in Japanese women.
——— endometrial cancer; breastfeeding; risk factor; case-control study

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Age-adjusted incidence rates for endometrial cancer have doubled during the past two decades among Japanese women. The rising incidence possibly may be due to changes in lifestyle, or changes in reproductive factors such as childbearing and contraception, as these characteristics have been associated with endometrial cancer risk in Western populations. In Western countries, there is considerable evidence that reproductive factors play a role in the etiology of endometrial cancer. Nulliparity and obesity have been associated with a higher risk, whereas oral contraceptive (OC) use has been associated with a lower risk (Kirschner et al. 1981; Kelsey et al. 1982; Zumoff 1982; Austin et al. 1991; Schapira et al. 1991; Brinton et al. 1992; Shu et al. 1992; Kalandidi et al. 1996; McPherson et al. 1996; Iemura et al. 2000; Herrinton et al. 2001). A few studies have examined the association between breastfeeding and endometrial cancer risk (Rosenblatt and Thomas 1995; Salazar-Martinez et al. 1999; Newcomb and Trentham-Dietz 2000); however, the findings from these studies are inconsistent.

The reproductive characteristics of Japanese women, however, are different from those of Western populations. For instance, 15%, 36%, and 59% of contraceptive-using women choose OCs in the United States, France, and Germany, respectively, whereas the prevalence of OC use is only 1.5% among Japanese women who use contraception. Only 1.8% of Japanese women older than 50 years have used hormone replacement therapy (HRT), whereas the prevalence of HRT usage is 53% among US women aged 50-59 years. These differences make it difficult to generalize findings obtained in Western studies to Japanese women. There have, however, been a few studies evaluating risk factors for endometrial cancer in Japanese women (Inoue et al. 1994; Hirose et al. 1996, 1999). Therefore, this study was undertaken to further characterize endometrial cancer risk factors in Japanese population.

Subjects and Methods

This case-control study was a collaborative investigation in three areas of Japan (Tokyo, Kanagawa, and Miyagi). Cases were accrued from three university hospitals from January 1, 1998, through December 31, 2000. Eligible cases included Japanese women between 20 and 80 years of age who underwent surgery for a diagnosis of endometrioid endometrial cancer confirmed by histology. The cases resided in defined geographic catchment areas, and had not received treatment previously. One hundred sixty seven cases were eligible for the study and 12 subjects refused to participate. Thus, 155 (93%) of the eligible cases participated. Stage distribution of the cases was as follows: stage I, n = 104; stage II, n = 14; stage III, n = 33; and stage IV, n = 4.

The controls were selected from women who attended gynecologic outpatient clinics in the university hospitals for cervical cancer screening. Controls included only women with intact uteri. Ninety six women were included as controls; however, 9 women refused participation (participation rate, 91%). Cases and controls were not matched in terms of age or other variables.

The protocol for this study was approved by the Ethics Committee at Tohoku University Graduate School of Medicine (Sendai, Japan).

Gynecologists interviewed the cases and controls using a standard questionnaire asking about demographic information, medical history, cigarette use, and reproductive history (parity, gravidity, and ages at first pregnancy, last delivery, menarche, menopause, and lactation). Body mass index (BMI) was calculated based on self-reports of weight (kg)/height $(m)^2$. The distribution of continuous variables was examined among cases and controls and divided into two or three categories.

To estimate the risk of endometrial cancer associated with various factors, we calculated age-adjusted and multivariate odds ratio (ORs) along with 95% confidence interval (CI) using unconditional logistic regression analysis. Statistical Analysis System (SAS Institute, Cary, NC, USA) software was used for all statistical analyses.

Results

The mean ages of cases and controls were 56.1 years and 49.6 years, respectively. Table 1 presents age-adjusted ORs and 95% CIs of the selected variables for the risk of endometrial cancer. Higher BMI was associated with higher risk (p = 0.01). OC use was associated with a lower risk of disease (OR, 0.16; 95% CI, 0.04-0.66), although only three cases and ten controls used OCs. Intra-uterine device use, history of HRT, smoking, sterility, hypertension, diabetes mellitus,

TABLE 1. *Baseline characteristics of cases and controls*

Caracteristics	Cases	%	Controls	%	Age-adjusted OR	95% CI	*p* value
Age (years)							
< 45	15	9.7	39	40.6			
45-55	52	33.6	23	24			
55-65	55	35.4	24	25			
≧ 65	33	21.3	10	10.4			
BMI (kg/m^2)							
< 20.04	36	23.3	26	27.1	1.00		
20.04-21.63	27	17.4	35	36.5	0.47	0.22-0.99	
21-64-23.92	45	29.0	20	20.8	1.24	0.58-2.67	
≧ 23.93	47	30.3	15	15.6	1.92	0.86-4.30	0.01
Oral contraceptive use							
Never	152	98.1	86	89.6	1.00		
Ever	3	1.9	10	10.4	0.16	0.04-0.66	0.01
IUD use							
Never	148	95.5	90	93.8	1.00		
Ever	7	4.5	6	6.2	0.54	0.17-1.71	0.29
HRT use							
Never	132	85.16	85	88.5	1.00		
Ever	23	14.84	11	11.5	1.4	0.63-3.14	0.41
Cigarette smoking							
Never	126	81.3	77	80.2	1.00		
Ever	29	18.7	19	19.8	1.30	0.65-2.61	0.52
Sterility							
Never	143	92.3	87	90.6	1.00		
Ever	12	7.7	9	9.4	0.81	0.31-2.11	0.66
Hypertension							
Never	115	74.2	87	90.6	1.00		
Ever	40	25.8	9	9.4	2.15	0.95-4.86	0.45
Diabetes mellitus							
Never	139	89.7	92	95.8	1.00		
Ever	16	10.3	4	4.2	1.82	0.56-5.92	0.32
Personal cancer history							
Never	139	89.7	92	96.8	1.00		
Ever	16	10.3	4	4.2	1.78	0.55-5.73	0.33

and personal cancer history were not associated with risk. There were 20 persons who had personal cancer history. Among them 11 persons had breast cancer and the remaining nine persons had cancer history at various sites, such as colon cancer, rectal cancer, thyroid cancer, gastric cancer, lung cancer, and ovarian cancer. Four of the 20 persons had hormone therapy.

Table 2 shows the ORs for the association of endometrial cancer with reproductive factors.

TABLE 2. *Multivariate Odds Ratio and 95%Confidence Intervals for Endometrial Cancers-According to Reproductive Factors*

Variables	Cases	%	Controls	%	OR*	95% CI	*p* value
Menopausal status							
Pre	51	32.9	55	57.3	1.00		
Post	104	67.1	41	42.7	0.91	0.39-2.14	0.82
Gravidity							
Never	20	12.9	9	9.4	1.00		
Ever	135	87.1	87	90.6	0.34	0.13-0.92	0.03
No. of pregnancies							
0	20	12.9	9	9.4	1.00		
1	27	17.4	16	16.7	0.43	0.14-1.33	
2	42	27.1	32	33.3	0.34	0.12-0.97	
≧ 3	66	42.6	39	40.6	0.29	0.10-0.85	0.04
Parity							
Never	36	23.2	21	21.9	1.00		
Ever	119	76.8	75	78.1	0.46	0.22-0.96	0.04
No. of deliveries							
0	36	23.2	21	21.9	1.00		
1	29	18.7	18	18.8	0.45	0.18-1.12	
2	68	43.9	44	45.8	0.47	0.21-1.04	
≧ 3	22	14.2	13	13.5	0.44	0.16-1.20	0.1
Age at first delivery**							
≦ 24	43	36	11	14.7	1.00		
25-26	36	30.3	23	30.7	0.45	0.18-1.10	
27-29	21	17.7	23	30.7	0.30	0.12-0.78	
≧ 30	19	16	18	24	0.35	0.13-0.96	0.05
Age at last delivery**							
≦ 25	23	19.3	6	8	1.00		
26-30	40	33.6	25	33.3	0.48	0.16-1.45	
31-33	39	32.8	26	34.7	0.45	0.15-1.36	
≧ 34	17	14.3	18	24	0.28	0.08-0.94	0.02

* OR adjusted for age, BMI, and oral contraceptive use.
** Parous women only.

The ORs were adjusted for age, BMI, and OC use. Gravidity was inversely associated with endometrial cancer risk. Women who reported ever being pregnant had only one third the risk of endometrial cancer compared with women who had never been pregnant (OR, 0.34; 95% CI, 0.13-0.92, $p = 0.03$). Women who reported three or more pregnancies had about one third the risk of women with no pregnancies (OR, 0.29; 95% CI, 0.10-0.85).

Parity was also inversely associated with endometrial cancer risk. Women who reported ever having delivery had about one half the risk of endometrial cancer compared with women who had never delivered (OR, 0.46; 95% CI, 0.22-0.96, $p = 0.04$). Higher age at the first or last deliveries was associated with a lower risk for endometrial cancer ($p = 0.05$, $p = 0.02$). Age at menarche, menopausal status, age at menopause, history of dysmenorrhea, and history of abortion were not associated with risk (data not shown).

Only parous women, representing 119 cases and 75 controls, were included in the analysis of the association between breastfeeding and endometrial cancer risk presented in Table 3. Table 3 also showed the age distribution of both cases and control and that of the lapse of the last breast feeding. The ORs were adjusted for age, BMI, and OC use as shown in Table 3. Compared with parous women who had never breastfed, the multivariate odds ratio for women who had ever breastfed was 0.37 (95% CI: 0.17-0.82, $p = 0.013$). A greater lapse of time since breastfeeding concluded was directly associated with an increased risk of endometrial cancer (OR of 20-29 years, 3.10, 95% CI: 1.14-8.48, and OR of 30 or longer, 3.85, 95% CI: 1.00-14.84, $p = 0.045$). Then, we analyzed the association between frequency or duration of breastfeeding and endometrial cancer risk, but did not find any significant association (data not shown).

Discussion

In this hospital-based case-control study among Japanese women, we observed a lower risk of endometrial cancer associated with OC use and gravidity, and a higher risk associated with higher BMI, older ages at first and last delivery and number of pregnancies. These findings were consistent with data obtained in prior Japanese studies (Inoue et al. 1994; Hirose et al. 1996, 1999). In contrast to the study by Inoue et al. (1994), our study failed to demonstrate an association between a history of hypertension, diabetes mellitus, or cancer.

Our study also demonstrated a reduction in the risk of endometrial cancer associated with breastfeeding. The proportion of never breastfeeding (35.3%) in endometrial cancer cases was larger than that in control, but the risk was signifi-

Table 3. *Multivariate Odds Ratio for Endometrial Cancers in relation to breastfeeding and age among parous women*

Variables	Cases (*n*)					Controls (*n*)					OR*	95% CI	*p* value
	Total	<45	45-55	55-65	65≦	Total	<45	45-55	55-65	65≦			
breastfeeding													
Never	42	1	10	24	7	11	3	3	2	3	1.00		
Ever	77	1	31	26	19	64	25	14	18	7	0.37	0.17-0.82	0.013
Years since last breastfed**													
1-19	12	1	9	1	1	33	24	7	2	0	1.00		
20-29	31	0	17	12	2	20	1	7	11	1	3.10	1.14-8.48	
≧30	34	0	5	13	16	11	0	0	5	6	3.85	1.00-14.84	0.045

* Adjusted for age, BMI and oral contraceptive use.
** Ever breastfed women only.

cant even after been adjusted for age, BMI, and contraceptive use. The risk reduction of endometrial cancer was associated not only with breastfeeding itself but also with time since the last breastfeeding. From 1982 to 2000, seven case-control studies conducted in six countries, including four developing countries, examined the relationship between breastfeeding and the risk of endometrial cancer. Four early studies, two of which were Japanese, failed to support an association (Kelsey et al. 1982; Brinton et al. 1992; Hirose et al. 1996, 1999). Three recent Western studies, however, suggested a protective effect of breastfeeding (Rosenblatt and Thomas 1995; Salazar-Martinez et al. 1999; Newcomb and Trentham-Dietz 2000). This effect was more pronounced with recent breastfeeding, diminishing as the history of breastfeeding became more remote (Rosenblatt and Thomas 1995; Newcomb and Trentham-Dietz 2000). Our findings were consistent with those of the latter studies, making this the first report that notes an inverse association between breastfeeding and the risk of endometrial cancer among Japanese women.

Exposure of the endometrium to estrogen in the absence of progesterone is thought to increase the risk of endometrial cancer (Key and Pike 1988- see comment for citation). In lactating women, the ovarian cycle is suppressed and blood estrogen levels are reduced (Baird et al. 1979). In the case of oral contraceptives, progesterone continually opposes estrogen, minimizing the duration of time the endometrium is exposed to unopposed estrogen. Thus, suppression of circulating estrogen levels, or opposition of estrogen by progesterone, may represent a biological mechanism accounting for the protective effects of pregnancy, oral contraceptives, and breastfeeding against carcinogenesis of endometrial tissue.

Among Japanese women, the birth rate decreased 28.1 to 9.3 per 1,000 during 1950-2000, and the proportion of women who exclusively breastfed decreased from 70.5% to 44.8% during the same period (Kaneda 2003). In our study, the proportion of women who breastfed for 13 months was 52.4%. The observed lower risk associated with breastfeeding in this study suggests that the recent increase in incidence of endometrial cancer in Japan may be in part attributed to a decrease in both the number of pregnancies and the prevalence of breastfeeding.

A limitation of this study was its lack of age matching. This resulted in a mean age of cases that was 6 years older than that of controls. It is unlikely that the lack of age matching resulted in serious distortion of our observations because all analyses were adjusted for continuous age. Furthermore, the findings in these studies were consistent with data obtained in several previous studies. Another limitation of the study was the small number of control. To overcome these limitations, in progress is our new case control study which matched ages of cases and controls and included two times more subjects of control. These data will confirm the present observations.

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Lymph-vascular space invasion and number of positive para-aortic node groups predict survival in node-positive patients with endometrial cancer☆

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Abstract

Objective. The aim of this study was to determine pathologic variables associated with disease-specific survival of node-positive patients with endometrial carcinoma treated with combination of surgery including pelvic and para-aortic lymphadenectomy and adjuvant chemotherapy.

Methods. Survival of 55 node-positive endometrial carcinoma patients prospectively treated with surgery and adjuvant chemotherapy between 1982 and 2002 at Hokkaido University Hospital was compared to various histopathologic variables. All patients underwent primary surgical treatment including pelvic and para-aortic lymphadenectomy followed by adjuvant chemotherapy consisting of intravenous cisplatin, doxorubicin, and cyclophosphamide. Survival analyses were performed by the Kaplan–Meier curves and the log-rank test. Independent prognostic factors were determined by multivariate Cox regression analysis using a forward stepwise selection.

Results. Among 303 consecutive endometrial cancer patients treated during the period of this study, 55 patients (18.2%), including 44 without peritoneal metastasis (FIGO stage IIIc) and 11 with peritoneal metastasis (FIGO stage IV), were found to have retroperitoneal lymph node metastasis. Multivariate Cox regression analysis revealed that peritoneal metastasis and lymph-vascular space invasion (LVSI) were independently related to poor survival in node-positive endometrial carcinoma. The estimated 5-year survival rate of stage IIIc patients with or without moderate/prominent LVSI was 50.9% and 93.3%, respectively with statistically significant difference ($P = 0.0024$). The estimated 5-year survival rate of stage IV patients was 20.0%. Prognosis of stage IIIc patients could be stratified into three groups by the number of positive para-aortic node (PAN) with an estimated 5-year survival rate of 86.4% for no positive PAN ($n = 23$), 60.4% for one positive PAN ($n = 13$), and 20.0% for ≥ 2 positive PAN ($n = 8$). The difference of survival rate between no or one positive PAN and ≥ 2 positive PAN was statistically significant ($P = 0.0007$ for no positive PAN vs. ≥ 2 positive PAN, $P = 0.0319$ for one positive PAN vs. ≥ 2 positive PAN). Multivariate analysis including number of positive PAN groups showed that LVSI, number of positive PAN groups were independent prognostic factors for survival. Survival of patients with stage IIIc disease could be stratified into three groups by combination of LVSI and number of positive PAN groups with an estimated 5-year survival rate of 93.3% for no or one positive PAN group with nil or minimal LVSI, 62.6% for no or one positive PAN group with intermediate or prominent LVSI, and 20.0% for ≥ 2 positive PAN groups irrespective of LVSI ($P = 0.0002$ for no or one positive PAN group with nil or minimal LVSI vs. ≥ 2 positive PAN groups, $P = 0.0223$ for no or one positive PAN group with nil or minimal LVSI vs. no or one positive PAN group with intermediate or prominent LVSI, $P = 0.0388$ for no or one positive PAN group with intermediate or prominent LVSI vs. ≥ 2 positive PAN groups).

Conclusions. LVSI and number of positive PAN groups were independent prognostic factors for stage IIIc endometrial cancer patients. Postoperative therapy and follow-up modality need to be individualized according to LVSI and the number of positive PAN for stage IIIc patients. New molecular markers to predict the prognosis of endometrial cancer patients preoperatively should be found for individualization

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of treatment. New chemotherapy regimen including taxane needs to be considered as an adjuvant therapy for patients with node-positive endometrial cancer.

Keywords: Endometrial carcinoma; Prognostic factor; Stage IIIc; Para-aortic lymph node metastasis; Lymph-vascular space invasion

Introduction

The incidence of endometrial carcinoma is rapidly increasing in Japan, with an estimated 4800 new cases in the year 2000; this number is still much lower than that in the United States, which was 40,100 in the year 2003 [1].

A majority of patients are diagnosed as being without clinical evidence of extrauterine spread, the International Federation of Gynecology and Obstetrics (FIGO) stages I and II, and have a 5-year survival of approximately 90%. Involvement of pelvic and para-aortic lymph nodes has been recognized as a poor prognostic factor in endometrial carcinoma. The new FIGO surgical staging system classifies endometrial carcinoma with metastasis to the pelvic and/or para-aortic lymph nodes as stage IIIc. The overall 5-year survival for patients with stage IIIc disease varies considerably, depending on the presence of various risk factors, extent of lymphadenectomy, and postoperative adjuvant therapy. Data on surgically staged endometrial cancer patients with nodal involvement have been limited [2–5]. The distribution of nodal disease and its importance in predicting survival are largely unknown, since various procedures have been used to assess para-aortic and pelvic nodes in endometrial cancer, that is, biopsies from enlarged nodes only, selective nodal sampling from multiple sites, pelvic lymphadenectomy, and para-aortic and pelvic lymphadenectomy. It is obvious that para-aortic and pelvic lymphadenectomy is most accurate of these methods. However, para-aortic and pelvic lymphadenectomy is not regarded as the standard surgical procedure for endometrial cancer because the therapeutic significance of the procedure has not yet been sufficiently demonstrated [6,7]. We have routinely performed complete lymphadenectomy in all patients with endometrial cancer because (i) nodal status is the most important prognosticator [2], (ii) results of lymphadenectomy allow tailoring of postoperative adjuvant treatment, (iii) there is an apparent small survival advantage after lymphadenectomy [8], and (iv) there is no increased morbidity with lymphadenectomy [9].

We have previously reported that para-aortic lymph node metastasis is an independent prognostic factor for endometrial cancer patients as well as cell type, grade and LVSI [10,11]. However, the fact that patients with para-aortic lymph node metastasis sometimes have shown long survival suggest that specific prognostic factors may exist for node-positive endometrial carcinoma.

In this study, we tried to identify the independent histopathologic prognostic factors for endometrial carcinoma patients with lymph node metastasis, who were uniformly treated with extensive surgical staging that includes modified radical hysterectomy and systematic pelvic and para-aortic lymphadenectomy followed by adjuvant chemotherapy. We also described the distribution of nodal disease in FIGO stage IIIc endometrial cancer and evaluated whether nodal distribution is related to survival.

Materials and methods

Patients

A total of 303 patients with endometrial carcinoma underwent primary radical surgical treatment from 1982 to 2002 at the Department of Obstetrics and Gynecology, Hokkaido University Hospital. Among 303 patients, 55 patients showed positive retroperitoneal lymph nodes. All subjects underwent modified radical hysterectomy, bilateral salpingo-oophorectomy, and systematic retroperitoneal lymphadenectomy which consisted of complete dissection of pelvic and para-aortic lymph nodes from the femoral ring to the level of the renal vein. All lymphatic tissues that surrounded the arteries and veins were completely removed. The FIGO (1988) stage of the patients was as follows: 44 (80%) stage IIIc and 11 (20%) stage IV. Stage IV disease with distant metastasis (liver or lung metastasis) was excluded from this analysis. We, therefore, defined stage IV patients as node-positive patients with peritoneal metastasis. Median follow-up period was 43 months (1–200 months). The patients' characteristics are shown in Table 1. All patients were treated with an adjuvant chemotherapy of CAP (cyclophosphamide: 350 mg/m^2, adriamycin: 40 mg/m^2 and cisplatin: 50–70 mg/m^2) every 3 weeks.

The following histopathologic prognostic factors were included in the survival analyses: FIGO (1988) stage, histologic subtype, depth of myometrial invasion, architectural grade (AG), nuclear grade (NG), LVSI, ovarian metastasis, PAN metastasis, and number of positive PAN group. All risk factors except number of positive PAN were determined as previously described [10,11]. We defined a node group by its laterality and location. Pelvic node groups include common iliac nodes, external iliac nodes, internal iliac nodes, obturator nodes, median deep inguinal nodesal deep inguinal nodes, parametrial nodes, and sacral nodes. PAN metastasis was investigated by dividing the metastatic group according to the sites above and below the inferior mesenteric artery and left and right sections bordering on the midline of the aorta as previously described [3]. In this analysis, caval (pre-, peri-, retro-) and aortocaval nodes were