Analysis of Immunohistochemical Characteristics and Recurrence After Complete Remission with Fertility Preservation Treatment in Patients with Endometrial Carcinoma and Endometrial a typical Hyperplasia

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Abstract

Objective To investigate the relationship between immunohistochemical characteristics and recurrence after complete remission(CR) with fertility preservation treatment in patients with endometrial cancer (EC) and endometrial atypical hyperplasia (AH).

Methods The clinical data and immunohistochemical results of 53 patients with EC and 68 patients with AH admitted to Peking University People's Hospital from January 2010 to January 2021 were retrospectively analyzed. Patients were divided into two groups according to whether recurrence after complete remission (CR): Group 1: recurrence after CR; Group 2: no recurrence after CR, for statistical analysis.

Results (1)The expression rate of ER in Group 1 was lower than that in Group 2, ($P < 0.05$). The expression rate of Ki-67 in Group 1 was significantly higher than that in Group 2, ($P < 0.01$). The expression rates of PR, P16, P53, and PTEN were not significantly different between the two groups ($P > 0.05$); (2) Combination index ER/ Ki-67 row ROC curve analysis, there was a significant difference (P < 0.01), the best cut-off value was 3.55, sensitivity 0.730, specificity 1.000, Youden index 0.730. The 3-year RFS of high rate patients was 100%, and that of low rate patients was 42.3%, P 0.01.

Conclusions The expression rate of Ki-67 is of great significance in predicting the recurrence of EC after fertility preservation therapy. The best cut-off value of combination index ER/ Ki-67 (3.55) was better than a single immunohistochemical marker in predicting recurrence of EC after fertility preservation treatment.

Background

Endometrial cancer (EC) is a major gynecological malignancy in developed countries. EC may develop from precursor lesions, mainly endometrial atypical hyperplasia (AH). 29% of untreated AH patients may develop into EC[1]. The main treatment for AH and EC is surgery. However, about 7.1% of the patients diagnosed with endometrial cancer in China are between 20 and 44 years old[2]. Therefore, surgery is not the best choice for young patients, especially those who want to maintain fertility. It is reported that the complete remission rate of progesterone-based fertility preservation treatment for endometrial cancer patients is 50-80%, and the pregnancy rate is 25-30%. However, the recurrence rate is as high as 24 - 40%[3-6]. This is one of the main causes of death. It is always the focus of clinical research to predict the risk factors of recurrence after fertility preservation treatment for endometrial cancer. Some studies suggest that tumor stage, histological grade, pathological type, depth of myometrial invasion, and lymphatic vascular space involvement are high-risk predictors of recurrence[7-10]. At present, there is no effective predictor for the recurrence of patients with fertility preservation treatment. Therefore, it is very important to explore more indicators related to recurrence and prognosis of EC. This study retrospectively analyzed the diagnosis, clinical data, and immunohistochemical characteristics of 53 cases of EC and 68 cases of AH in our hospital. The aim was to analyze and summarize the relationship between the immunohistochemical characteristics of EC and AH patients in our hospital and the recurrence after fertility preservation treatment, to provide the relevant theoretical basis for the fertility preservation treatment of EC patients in the future.

Methods

Medical Records

Methods 53 patients with EC and 68 patients with AH were collected from Peking University People's Hospital from January 2010 to January 2021. The indications were: (1) The age is less than 40 years old, and 40-45 year old patients can fully inform the risk and fully informed consent; (2) Pathological tissue types are endometrial atypical hyperplasia or high differentiation (G1) endometrioid carcinoma, which can be included in the assessment and full knowledge of G2 patients;(3) Imaging examination confirmed that the tumor was confined to the endometrium, and patients with superficial myometrial invasion could also be included after evaluation and full knowledge; (4) There was no contraindication of progesterone treatment and pregnancy; (5) The fertility function was evaluated before treatment, and there were no other fertility obstacles; (6) Signing the informed consent and having a good follow-up condition[11].

Condition assessment before treatment

They include history collection, physical examination, and general condition assessment, hysteroscopy, and direct biopsy to obtain endometrial specimens and do immunohistochemistry, ultrasound, or pelvic MRI to evaluate muscle infiltration and metastasis, and to assess metabolic syndrome.

Treatment plan and monitoring

Oral administration of medroxyprogesterone (MPA) or megestrol acetate (MA) is the first choice. MPA daily 250~500 mg or MA 160~320 mg.

When the first choice of drug treatment did not achieve complete remission. The following drugs can be selected or combined to continue treatment: (1) gonadotropin-releasing hormone agonist (GnRH-a), 3.75 mg, subcutaneous injection once every 28 days; (2) levonorgestrel-releasing intrauterine system (LNG-IUS). For patients who can not tolerate high-dose progesterone systemic therapy, LNG-IUS and GnRH-a can be used together.

Initial hysteroscopy biopsy or diagnostic curettage, endometrial pathology, and immunohistochemistry, including ER, PR, P16, P53, PTEN, Ki-67, and so on. After treatment, once every 4~6 weeks, the endometrial thickness and myometrial invasion were observed by transvaginal color Doppler ultrasound. Endometrial tissues were collected under hysteroscopy every 3 months for pathological examination of the endometrium. Due to the time span and the initial diagnosis of patients, some patients' immunohistochemical data were missing.

Efficacy evaluation criteria (AH efficacy evaluation refer to EC standard): (1) Complete remission (CR): there was no lesion of endometrial atypical hyperplasia and endometrioid carcinoma in histopathology. (2) Partial remission (PR): the degree of congestion of endometrial glands decreases, but the nipple and sieve-like structures can still exist, and the atypia of glandular epithelium is reduced. (3) No response (NC): compared with the pathological results before treatment, there was no change in cancer tissue. (4) Progression of disease (PD): the histopathological grade of the tumor increased and the atypia of cells increased. (5) Recurrence: after complete remission, the lesion before treatment (endometrial atypical hyperplasia or endometrioid carcinoma) appeared again.

Grouping

According to the recurrence after complete remission (CR) of AH and EC patients, the patients were divided into group 1: recurrence after CR group. Group 2: no recurrence after CR group, statistical analysis.

Statistical methods

SPSS 25.0 software was used for statistical analysis. The counting data were expressed as cases and percentages. X^2 test or Fisher's exact probability method was used for comparison between groups; The measurement data are normally expressed in normal distribution and expressed in $\bar{x}\pm s$. test or Fisher's exact probability method was used for comparison between groups; The measurement data are normally expressed in normal distribution and expressed in terms of $M(P_{25}, P_{75})$. The mean values of the two groups were compared with the nonparametric Mann-Whitney U test. Taking P 0.05 as a standard with the significant difference in Statistics. Receiver operating characteristic curve analysis (ROC) and the Youden index was used to calculate the best critical value. The area under the curve (AUC) represents the validity of various indicators for predicting recurrence. Youden index = [sensitivity + specificity] - 1, and the maximum value is the best critical value. Kaplan–Meier curve was used to describe the RFS.

Results

Efficacy and outcome

Of the 68 AH patients, 55 cases reached CR (CR rate was 80.9%), 5 cases (7.4%) PR, 7 cases (10.3%) PD, 1 cases (1.5%) gave up fertility-sparing treatment, and 6 cases recurrence after CR (recurrence rate was 8.8%).Of the 53 EC patients, 42 cases reached CR (CR rate was 79.2%), and 6 cases (11.3%) PR. 1 cases (1.9%) PD, 1 cases (1.9%) NC, 2 cases (3.7%) gave up fertility-sparing treatment, 1 cases (1.9%) were lost, 9 cases had recurrence after CR (the recurrence rate was 17%). See Table 1.

Table 1

Grouping and fertility preservation treatment (n)

	Group1recurrence after CR	Group 2no recurrence after CR	PR	NC	PD	Give up treatment	Lost to follow up
AH	6	49	5	0	7	1	0
EC	9	33	6	1	1	2	1
Total	15	82	11	1	8	3	1

Abbreviations: AH, atypical hyperplasia; EC, endometrial cancer; CR, complete remission; PR, partial remission; NC, no response; PD, progression of the disease.

Relationship between clinicopathological characteristics, treatment, and efficacy

The average age of 97 patients in the two groups was 31.76±5.37 years, of which 3 patients were older than 40 years old. The average BMI was 26.94±5.06kg/m2, Among these, 61 patients (63%) had BMI > 25 kg/m2, (36 patients (37%) had BMI < 25 kg/m2, 35 patients (36%) had BMI > 25, < 30 kg/m2. 17 patients (18%), BMI was more

than 30, < 35 kg/m2, and 9 patients (9%) had BMI > 35 kg/m2. 22 cases were complicated with polycystic ovary syndrome (PCOS), 8 cases with diabetes mellitus (DM). There were no significant differences in age, BMI, complications, tumor histological grade, FIGO stage, and treatment methods between the two groups (P 0.05). See Table 2.

Table 2
Clinical data predict the efficacy of fertility preservation treatment

Characteristics		Group 1	Group2	P-value
Age(years)($\bar{x}$ ±s)		30.20 ± 4.52	32.05 ± 5.48	0.222
BMI (kg/m2)($\bar{x}$±s)		28.08 ± 5.14	26.73 ± 5.05	0.174
PCOS(n)		3	19	1.000
Diabetes mellitus (n)		1	7	1.000
EC Grade(n)				1.000
	G1	8	27	
	G2	1	6	
EC FIGO(n)				1.000
	Ia	8	29	
	Ib	1	4	
Therapeutic method (n)				0.819
	MA	3	12	
	MPA	5	39	
	MPA + GnRH-a	3	14	
	MPA + LNG-IUS	1	5	
	MPA + GnRH-a + LNG-IUS	3	12	

Abbreviations: BMI, body mass index; PCOS, polycystic ovarian syndrome; FIGO, International Federation of Gynecology and Obstetrics; MA, megestrol acetate; MPA, medroxyprogesterone; GnRH-a, gonadotropin-releasing hormone agonist; LNG-IUS, levonorgestrel-releasing intrauterine system. Note:$\bar{x}$±s is the average ± Standard deviation.

Relationship between immunohistochemistry and efficacy

The expression rate of ER in group 1 was significantly lower than that in group 2 (P 0.05), the expression rate of Ki-67 in group 1 was significantly higher than that in group 2 (P 0.01), but the expression rates of PR, p53, p16, and PTEN had no significant difference (P 0.05); The same results were found in AH or EC subgroups, as shown in Table 3.

Table 3

Immunohistochemical （IHC）predict recurrence after conservation therapy

IHC	Total		*P*-value	AH		*P*-value	EC		*P*-value
	Group1	Group 2		Group 1	Group 2		Group 1	Group 2	
ER [*M(P25,P75)*]	60 （50,70）	80 （62.5,90）	0.015	65 （17.5,90）	70 （57.5,90）	0.476	60 （50,65）	80 （70,82.5）	0.004
PR [*M(P25,P75)*]	75 （50,90）	90 （50,90）	0.464	75 （38.75,90）	90 （65,90）	0.221	70 （50,90）	75 （47,90）	0.840
P16+ [% (n/N)]	71.4 （5/7）	80.5 （33/41）	0.625	75（3/4）	82.6 （19/23）	1.000	66.7 （2/3）	77.8 （14/18）	1.000
P53+ [% (n/N)]	58.3 （7/12）	36.0 （18/50）	0.198	40（2/5）	41.7 （10/24）	1.000	71.4 （5/7）	30.8 （8/26）	0.084
PTEN+ [% (n/N)]	62.5 （5/8）	26.2 （11/42）	0.092	50（2/4）	26.1 （6/23）	0.558	75 （3/4）	26.3 （5/19）	0.103
Ki-67 [*M(P25,P75)*]	30 （22.5,30）	10（5,20）	0.000	30 （28.75,35）	10（5,25）	0.001	30 （20,30）	10（5,10）	0.001

Abbreviations: IHC, immunohistochemical; AH, atypical hyperplasia; EC, endometrial cancer; ER, estrogen receptor; PR, progesterone receptor. Note（*M*(P_{25}, P_{75}) represents the median (25th-75th percentile);n represents the number; N represents the effective number of cases

ROC curve (receiver operating characteristic curve analysis) showed that AUC values of ER, PR, p53, Ki-67, and ER / Ki-67 were 0.677, 0.519, 0.365, 0.114, and 0.908 respectively, and AUC of ER / Ki-67 combined ratio was the highest (Fig. 1). The best critical value of combined ratio was 3.55 (n = 97, AUC = 0.908, sensitivity 73.5%, specificity 100.0%, Youden index 0.735, (*P*＜0.01)). According to the cut-off value, patients with a combined ratio of more than 3.55 were defined as the high ratio group, and other patients were defined as the low ratio group. The 3-year RFS of high rate patients was 100%（95% confidence interval [CI] 100%–100%）, and that of low rate patients was 42.3%（95% confidence interval [CI] 19.6%–65.0%）, *P*＜0.01.

Discussion

With the development of molecular biology, immunohistochemical markers play an important role in the evaluation model of endometrial cancer recurrence. As the nuclear receptors of estrogen and progesterone, ER and PR regulate gene expression by activating and inhibiting transcription. The loss of expression is related to the invasiveness of the tumor[12]. The study[12] showed that the positive expression of PR was related to the histological grade and myometrial invasion. The expression rate of ER and PR decreased significantly in EC patients with high grade and deep myometrial invasion. Ki-67 as a nuclear antigen related to cell growth and development is recognized as a marker of cell proliferation. It reflects the proportion of malignant cells and is related to tumor progression,

metastasis, and prognosis[13]. High Ki-67 is associated with poor prognosis in esophageal, colorectal, pancreatic, and other cancers[14-16]. In female malignant tumors, the critical value of Ki-67 has been used as an important prognostic indicator of breast cancer. In endometrial carcinoma, Ki-67 also shows its potential value. Tumor suppressor gene p53 encodes nuclear phosphorylation protein, which can regulate transcription, inhibit cell growth and induce apoptosis. P53 gene mutation can lose the normal tumor suppressor effect and promote malignant transformation of cells[17].

In 2013, TCGA project divided endometrial cancer into four molecular subtypes: POLE-mutated (7%),microsatellite instable (MSI) (28%), copy number low(39%), copy number high(26%)[12]. It is considered that[18] the prognosis of patients with POLE mutation is better. MSI is the characteristic genetic marker of Lynch's syndrome. The prognosis of copy number high patients is poor. However, TCGA typing is based on high-throughput deep sequencing, which integrates genomics, transcriptomics, proteomics, gene copy number, and methylation data, with high economic cost and long time consuming; In addition, the data included in TCGA classification did not include clear cell carcinoma, undifferentiated carcinoma / dedifferentiated carcinoma, carcinosarcoma and other histological types. Therefore, this classification system is not feasible in clinical diagnosis at present.

Therefore, immunohistochemical staining is still an economical, convenient, and time-saving method in clinical practice. ER, PR, Ki-67, and p53 are closely related to the prognosis of EC patients, Studies show that[19] the best cut-off value of Ki-67 in predicting endometrial cancer recurrence is 38%. Patients with positive ER and PR had a better prognosis, while those with p53 mutation and high Ki-67 had a poorer prognosis. and the optimal cut-off value of ([ER + PR] / [p53 + Ki67]) was 0.92, which was a more statistically significant predictor than a single index[20]. And the combination of ER, PR, Ki-67, and P53 has been proved to be a statistically significant predictor of lymph node metastasis[13]. However, there is no study to show the relationship between IHC and recurrence of endometrial cancer patients treated with fertility-sparing treatment. In clinical treatment, it is necessary to evaluate the prognosis of patients treated with fertility-sparing treatment., which is the purpose of this study. Because of the small number of patients with fertility-sparing treatment, the study included 53 patients with EC and 68 patients with AH.

There was no significant difference between PR, p53, p16, PTEN, and the recurrence of fertility-sparing treatment patients. There was a significant difference between ER and recurrence (P 0.05). There was a significant difference between Ki-67 and recurrence (P 0.01). ROC curve showed that the AUC of combined ratio (ER / Ki-67) was the highest. We found the best critical value of 3.55 by the Youden index, which ensures the highest specificity and sensitivity. N = 97, AUC = 0.908, sensitivity 73.5%, specificity 100.0%, Youden index 0.735 (P < 0.01). This confirmed that the combined ratio was more predictive of prognosis than the expression level of a single IHC. It avoids the sidedness and inaccuracy of predicting recurrence from a single IHC.

The shortcomings of this study are as follows: First. The retrospective design of a single institution, and the number of EC patients treated with fertility-sparing treatment is small, resulting in a small number of samples. Second: the IHC index is affected by subjective factors, such as large differences in pathological staining, less endometrial collection, and the influence of pathological experts. The above deficiency may be the cause of no significant difference in age, BMI, and recurrence. It also leads to the situation that the specificity of the combination ratio is 100.0% at the optimal critical value and the 3-year RFS of high rate patients was 100%,

In conclusion, we combined the clinical characteristics of patients with immunohistochemical markers to explore the factors associated with the recurrence of fertility-sparing treatment and confirmed that the ratio of ER/Ki-67 is more effective in predicting prognosis than single IHC expression. This provides new suggestions for our clinical

diagnosis and treatment. Is it possible to add the evaluation of immunohistochemical markers before using fertility-sparing treatment in future clinical work? If the ratio of ER / Ki-67 is too high, it may indicate the poor prognosis of the disease, and it also indicates that the patient is prone to recurrence.

Declarations

Conflict of interest

The authors declared no potential conficts of interest with respect to the research, authorship and/or publication of this article.

Ethical approval

This study was approved by Institutional Review Board of Peking university people' hospital.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Figures

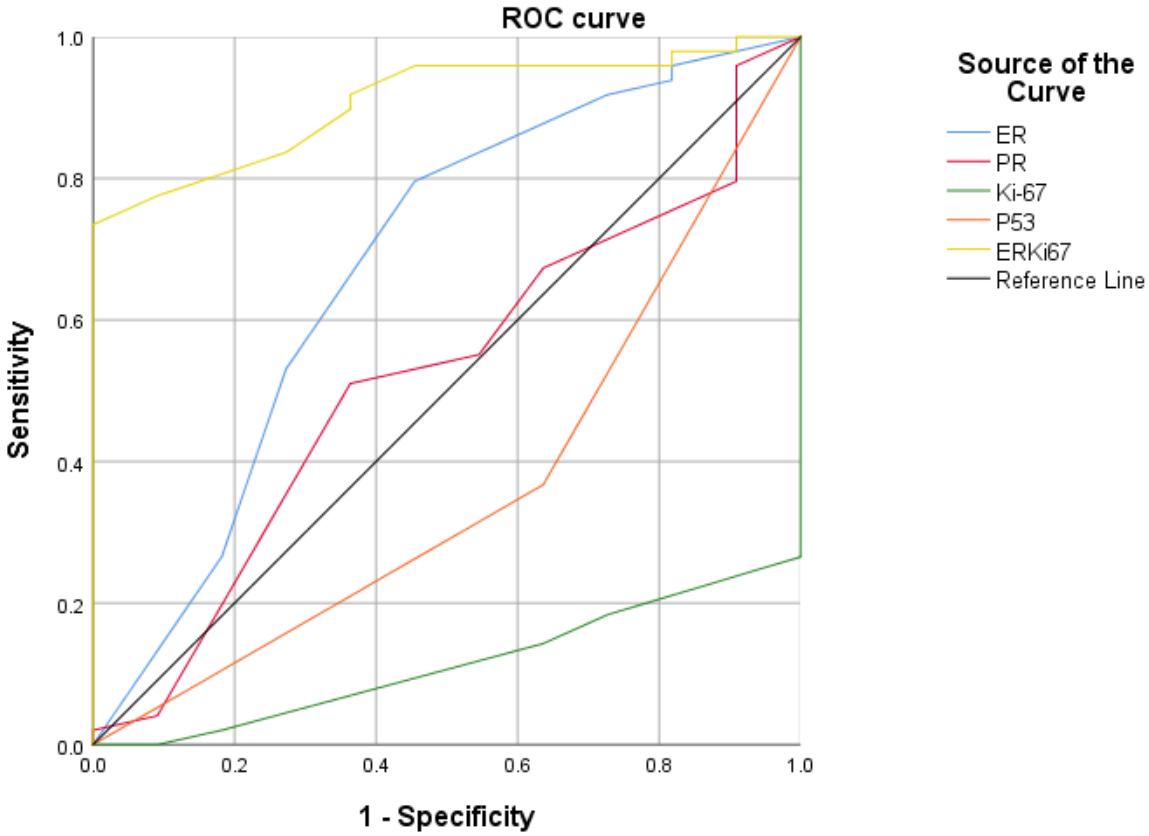

Figure 1

ROC curve (receiver operating characteristic curve analysis) showed that AUC values of ER, PR, p53, Ki-67, and ER / Ki-67 were 0.677, 0.519, 0.365, 0.114, and 0.908 respectively, and AUC of ER / Ki-67 combined ratio was the highest (Fig. 1).

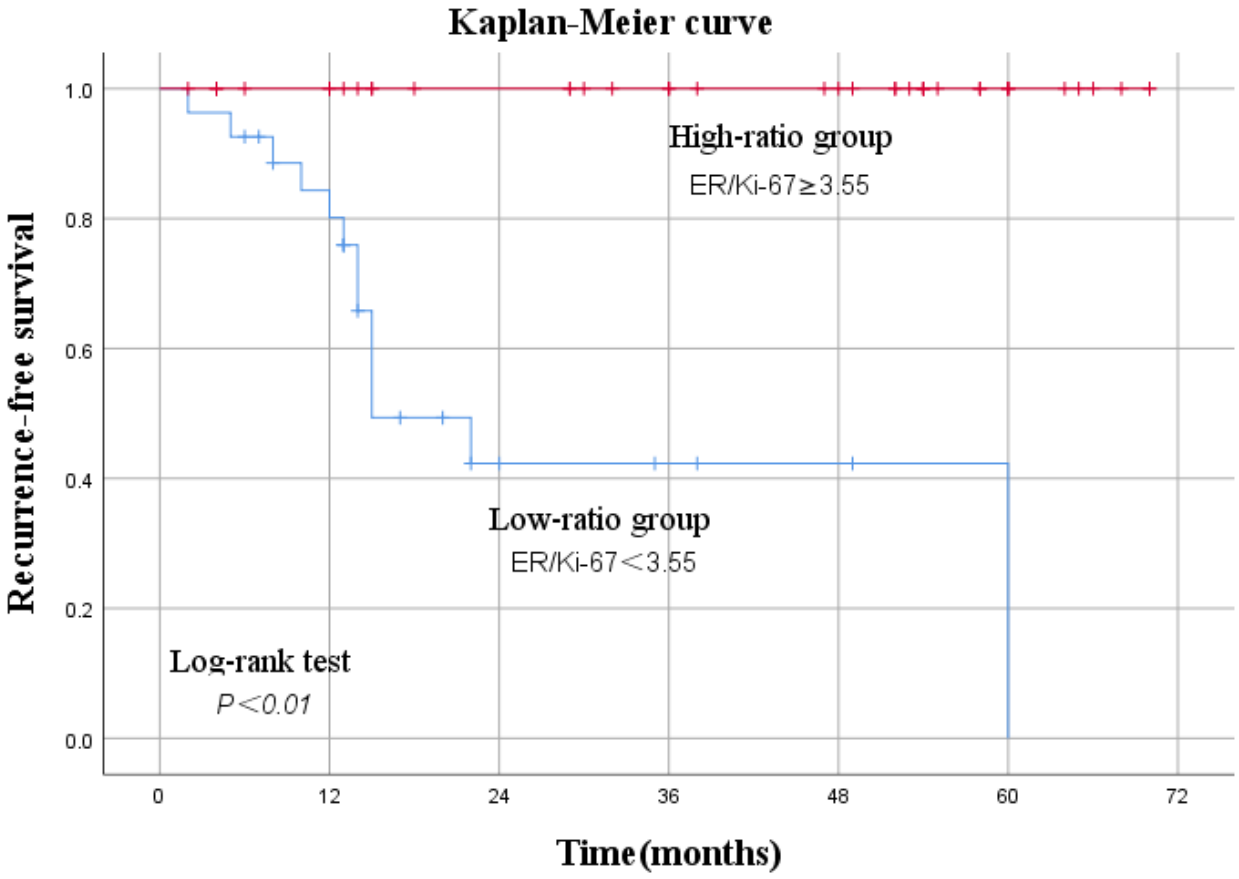

Figure 2

The area under the curve (AUC) represents the validity of various indicators for predicting recurrence. Youden index = [sensitivity + specificity] - 1, and the maximum value is the best critical value. Kaplan–Meier curve was used to describe the RFS.