Peritumoral and Intratumoral Lymphocytic Infiltration and Stromal Reaction in High-Risk Endometrioid Carcinomas

Yüksek Riskli Endometrioid Karsinomlarda Peritümöral ve İntratümöral Lenfositik İnfiltrasyon Ve Stromal Reaksiyon

Mine Özşen[1], Fatma Öz Atalay[1], Birol Ocak[2], Seçil Hasdemir[1]

[1]Department of Pathology, Uludag University Faculty of Medicine, Bursa, Turkiye
[2]Department of Medical Oncology, Faculty of Medicine, Uludag University, Bursa, Turkiye

ABSTRACT

Objective: This study aims to evaluate the relationship between the local immune response such as peritumoral and intratumoral lymphocytic infiltration and stromal reaction, with prognosis and other clinicopathological parameters in high-risk endometrioid carcinomas.
Method: Totally 98 high-risk cases diagnosed as endometrioid carcinoma in our center between 2005 and 2017, were retrieved from the pathology archives and re-evaluated.
Results: Totally 98 cases diagnosed as high-risk endometrial carcinoma were evaluated. Intratumoral and peritumoral lymphocytic infiltration was compared to overall survival period and overall survival was significantly higher in tumors showing peritumoral and intratumoral lymphocytic infiltration ($p=0.000 <\alpha=0.05$, $p=0.008 <\alpha =0.05$). However, a similar relationship between stromal reaction and overall survival rate was not detected. Intratumoral and peritumoral lymphocytic infiltration was compared to recurrence-free survival and recurrence-free survival period was significantly higher in cases showing either type of lymphocytic infiltration ($p=0.003 <\alpha =0.05$, $p=0.050 <\alpha=0.05$).
Conclusions: Presence of peritumoral and intratumoral lymphocytic infiltration positively affects prognosis, similar to results of other studies. These parameters are important and necessary in predicting the prognosis of the patients and they should be included in pathology reports.

Keywords: Endometrioid carcinoma, intratumoral, high grade, lymphocytic infiltration, peritumoral

Received: 08.03.2021 **Accepted:** 09.05.2021

ÖZET

Amaç: Bu çalışma, yüksek riskli endometrioid karsinomlarda peritümöral ve intratümöral lenfositik infiltrasyon ve stromal reaksiyon gibi lokal immün yanıtın prognoz ve diğer klinikopatolojik parametreler ile ilişkisini değerlendirmeyi amaçlamaktadır.
Yöntem: Merkezimizde 2005-2017 yılları arasında endometrioid karsinom tanısı alan yüksek riskli 98 olgu patoloji arşivinden çıkarılarak yeniden değerlendirildi.
Bulgular: Yüksek riskli endometrial karsinom tanısı alan 98 olgu değerlendirildi. İntratümöral ve peritümöral lenfositik infiltrasyon, genel sağkalım süresi ile karşılaştırıldı ve genel sağkalım, tümör içi ve tümör içi lenfositik infiltrasyon gösteren tümörlerde anlamlı olarak daha yüksekti ($p=0.000 <\alpha=0.05$, $p=0.008 <\alpha =0.05$). Bununla birlikte, stromal reaksiyon ile genel sağkalım oranı arasında benzer bir ilişki saptanmadı. İntratümöral ve peritümöral lenfositik infiltrasyon, nükssüz sağkalım ile karşılaştırıldı ve nükssüz sağkalım süresi her iki tipte lenfosit infiltrasyonu gösteren olgularda anlamlı olarak daha yüksekti ($p=0,003 <\alpha =0,05$, $p=0,050 <\alpha=0,05$).
Sonuç: Peritümöral ve intratümöral lenfositik infiltrasyonun varlığı, diğer çalışmaların sonuçlarına benzer şekilde prognozu olumlu yönde etkiler. Bu parametreler hastaların prognozunu öngörmede önemli ve gereklidir ve patoloji raporlarında yer almalıdır.

Anahtar Sözcükler: Endometrioid karsinom, intratümöral, yüksek derece, lenfositik infiltrasyon, peritümöral

Geliş Tarihi: 03.08.2021 **Kabul Tarihi:** 05.09.2021

ORCID IDs: M.Ö.0000-0002-5771-7649, F.Ö.A.0000-0002-7188-6115, B.O.0000-0001-7537-1699, S.H.0000-0003-1769-7484

Address for Correspondence / Yazışma Adresi: Mine Özşen, MD Department of Pathology, Uludag University Faculty of Medicine, Bursa, Turkiye E-mail: m.isikoglu@hotmail.com

doi:http://dx.doi.org/10.12996/gmj.2023.31

INTRODUCTION

Endometrial carcinomas have a rapidly increasing incidence worldwide and are among the most common malignancies in women, in developed countries (1). The pathological prognostic parameters of endometrial carcinomas include histological type, histological grade, depth of myometrial invasion, lymphovascular invasion, lower uterine segment and cervical stromal involvement, serosal and adnexial involvement, lymph node metastasis and tumor stage (2,3). Endometrial carcinomas of grade 3 or any grade of tumors with lymphovascular invasion or invasion of more than half of the myometrium, stage 2,3 or 4 tumors and non-endometrioid tumors (serous carcinoma, clear cell carcinoma, dediferentiated carcinoma, undifferentiated carcinoma and carcinosarcoma) are defined as high-risk endometrial tumors. High-risk tumors comprise 15% of all endometrial cancers and have a poor prognosis (4,5).

Along with the introduction of immunotherapy as one of the modern treatment modalities which aim to activate immune response against tumor cells, the relationship between prognosis and local immune response (peritumoral lymphocytic infiltration, intratumoral lymphocytic infiltration) in various cancers has gained importance. It has been stressed out that the pathology reports should include local immune response as a pathological prognostic parameter (6-9).

In this study, we aimed to evaluate the relationship of the local immune response such as peritumoral and intratumoral lymphocytic infiltration and stromal reaction with prognosis and other clinicopathological parameters in high-risk endometrioid carcinomas.

MATERIALS and METHODS

This study includes 98 high-risk carcinomas, diagnosed as endometrioid carcinoma between 2005 and 2017 in our center. Follow-up periods and survival status of the patients were obtained from the hospital database. Patient age, tumor size, cervical stromal involvement, adnexal involvement and lymph node metastasis were acquired from the pathology reports. Hematoxylin-eosin-stained tissue slides were re-evaluated in terms of histological grade, myometrial invasion, lymphovascular invasion, infiltration pattern, stromal reaction, peritumoral lymphocytic infiltration, intratumoral lymphocytic infiltration and presence of necrosis.

Histological grading was performed according to International Federation of Gynecology and Obstetrics (FIGO) grading system. Depth of myometrial invasion was evaluated in two groups of less than half (superficial) or more than half (deep) of the myometrium, in the area with the deepest tumor invasion. Stromal reaction was categorized as 4 groups; absent, fibromyxoid, desmoplastic and mixed-type. Infiltration pattern categorized as infiltrative, expansive, MELF pattern and mixed (infiltrative and expansive). Intratumoral lymphocytic infiltration was evaluated in 2 groups as present or absent, considering clearly visible lymphocytes between/on tumor cells in 400x magnification. Peritumoral lymphocytic infiltration was evaluated similartly in 2 groups as present or absent, considering clearly visible lymphocytes between/on tumor cells in 400x magnification. Necrosis was evaluated in 4 groups as absent, present, mixed-type (with comedonecrosis) and comedonecrosis-only.

Ethical statement

Our study was conducted in accordance with the 1964 Helsinki declaration. The clinical research ethics committee of the University Faculty of Medicine approved the study (Approval number: 2020-21/9).

Statistical analysis

Frequency and percentage values for grade, myometrial invasion, lymphovascular invasion, stromal reaction, invasion pattern, intratumoral lymphocytic infiltration, peritumoral lymphocytic infiltration, necrosis, cervical stromal involvement, adnexial metastasis, type of surgery, stage, survival, presence of recurrence and choice of treatment is given. Normality assumptions for overall survival, recurrence-free survival, age, and tumor size were examined using the Kolmogorov-Smirnov tests. Variables were determined to have a normal distribution. Independent Samples t-test was then used for the analysis of variables with two groups, and ANOVA-F test was used for the analysis of variables with more than two groups. In addition, the relationship between categorical variables was examined using chi-square analysis. IBM-SPSS-21 was used for all the analyzes in the study.

RESULTS

A 98 cases of high-risk endometrial carcinoma diagnosed between 2005 and 2017 were determined (Table 1). Patient age varied between 40 and 84 with a mean age of 60,50±9,43. Mean tumor size was 4,81±2,11 cm, varying between 2 - 12,5 cm.

FIGO grade 2 was detected in 40 (40,82%), grade 3 in 35 (35,71%) and grade 1 in 23 (23,47%) cases. All cases diagnosed with grade 1 endometrial carcinoma were evaluated the high-risk endometrioid carcinomas because they were stage 2, 3, 4 or invasion of more than half of the myometrium. Stage 1 (with invasion of more than half of the myometrium) was detected in 4, stage 2 in 3, stage 3 in 12 and stage 4 in 4 cases. Myometrial invasion was less than half in 36 cases (36,73%) and more than half in 62 (63,27%). Lymphovascular invasion was not seen in 39 cases (39,8%) and was present in 59 cases (60,2%). Invasion patterns of the tumours was infiltrative in 73 (74,49%), mixed in 14 (14,29%) and expansive in 11 cases (11,22%). MELF pattern was present in 26 (27,1%) cases. Necrosis was absent in 33 cases (33,67%), present in 14 cases (14,29%), mixed in 9 (9,18%), comedo-type in 42 (42,86%).

Intratumoral lymphocytic infiltration was present in 28 cases and absent in 70 cases (71,43%). Peritumoral lymphocytic infiltration, on the other hand, was present in 45 (45,92%) cases, focal in 18 (18,37%) and absent in 35 (35,71%) (Figure 1). Stromal reaction was not observed in 18 cases (18,37%). Desmoplastic type stromal reaction was present in 50 (51,02%), fibromyxoid type reaction in 18 (%18,37) and mixed type in 12 (%12,24) cases (Figure 2).

Table 1. Frequency and Percentage Distributions of the Variables

	Variables	Frequency (n)	Percentage (%)
Grade	1	23	23,47
	2	40	40,82
	3	35	35,71
Myometrial invasion	<1/2	36	36,73
	>1/2	62	63,27
Lymphovascular invasion	Absent	39	39,80
	Present	59	60,20
Stromal reaction	Absent	18	18,37
	Fibromixoid stroma	18	18,37
	Desmoplastic stroma	50	51,02
	Mixed-type stroma	12	12,24
Infiltration pattern	Infiltrative	73	74,49
	Expansive	11	11,22
	Mixed-type	14	14,29
Intratumoral lymphocytic infiltration	Absent	70	71,43
	Present	28	28,57
Peritumoral lymphocytic infiltration	Absent	35	35,71
	Present	45	45,92
Necrosis	Focal	18	18,37
	Absent	33	33,67
	Necrosis	14	14,29
	Komedonekroz	42	42,86
	Mixed-type	9	9,18
Cervical stromal involvement	Absent	80	81,63
	Present	18	18,37
Adnexal involvement	Absent	77	78,57
	Present	21	21,43
Surgery	Total hysterectomy with bilateral salpingo-oophorectomy	4	4,30
	Total hysterectomy with bilateral salpingo-oophorectomy and bilateral pelvic lymph node dissection	90	95,70
Stage	1A G3	4	4,26
	1B G3	13	13,83
	3a	11	11,70
	3b	10	10,64
	3c	45	47,87
	4	11	11,70
Recurrence	Absent	71	72,45
	Present	27	27,55
Exitus	Absent	35	35,71
	Present	63	64,29
Treatment	Monitoring	7	7,61
	Post operative brachytherapy	10	10,87
	Post operative external radiotherapy	2	2,17
	Chemotherapy	5	5,43
	Chemoradiotherapy	43	46,74
	Brachytherapy and External radiotherapy	25	27,17

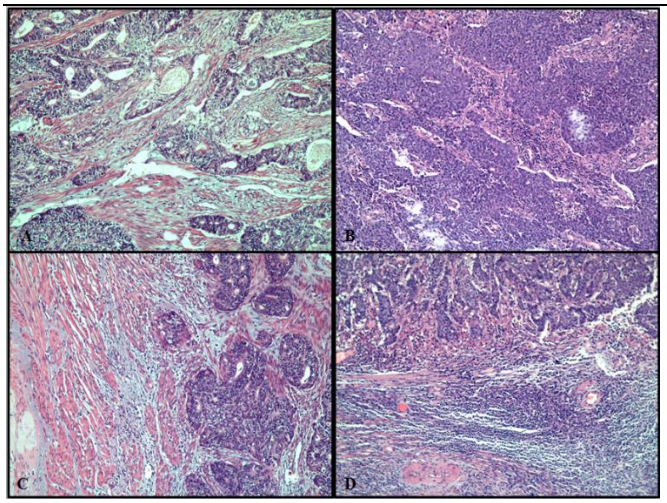

Figure 1. A. Intratumoral lymphocytic infiltration were not observed clearly between or on the tumor cells (H&E, x200) B. Intratumoral lymphocytic infiltration were observed between or on the tumor cells (H&E, x200) C. Peritumoral lymphocytic infiltration were not observed clearly around the tumor marjin (H&E, x200) D. Peritumoral lymphocytic infiltration were observed around the tumor marjin (H&E, x200)

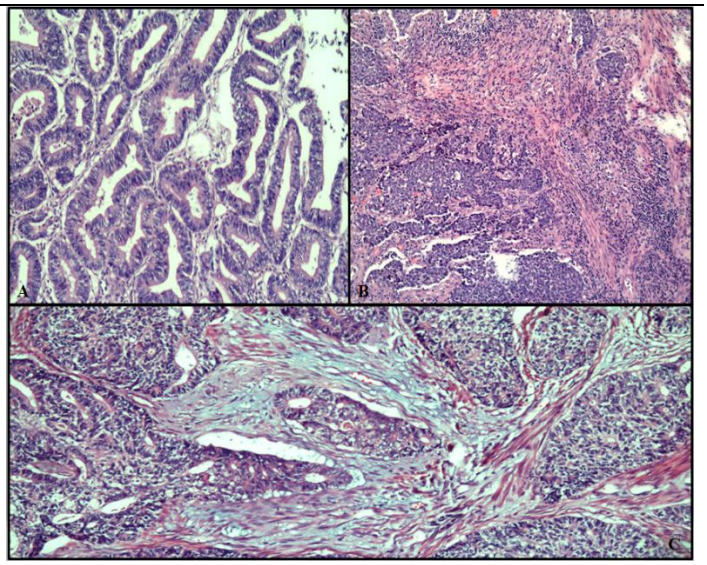

Figure 2. A. There is no stroma in the tumor (H&Ex200) B. Desmoplastic stroma (H&E, x100) C. Fibromixoid stroma (H&E, x200)

Intratumoral and peritumoral lymphocytic infiltration was compared to overall survival period and overall survival was significantly higher in tumors showing peritumoral and intratumoral lymphocytic infiltration (p=0.000 <α=0.05, p=0.008 <α =0.05). However, a similar relationship between stromal reaction and overall survival rate was not detected (Table 2). Intratumoral and peritumoral lymphocytic infiltration was compared to recurrence-free survival and recurrence-free survival period was significantly higher in cases showing either type of lymphocytic infiltration (p=0.003 <α =0.05, p=0.050 <α=0.05) (Table 3). When compared to other clinicopathological parameters, there was no statistically significant relationship between peritumoral or intratumoral infiltration or stromal reaction and any other parameter, other than myometrial invasion. The depth of myometrial invasion was found to be less in tumors showing peritumoral lymphocytic infiltration (p=0.035 <α=0.05).

Cervical stromal involvement was present in 18 (18,37%), adnexal involvement in 21 (21,43%) and metastasis (lymph node and/or distant organ) in 27 cases (27,55%). In 98 patients, the average follow-up period was 61,09, varying between 1 to 192 months. During the follow-up period, 35 (35,7%) patients died due to disease and 63 (64,3%) were alive and well. The average recurrence-free survival period was 59,26±41,31 months and the overall survival period was 68,35±40,65 months.

Table 2. T test/ANOVA F test analysis of variables of total survival and stromal reaction, intratumoral and peritumoral lymphocytic infiltration variables

	Variables	Average	Standard Deviation	F / t	p value
Stromal reaction	Absent	79,28	28,64	1,450 / -	0,233
	Fibromixoid stroma	53,28	30,48		
	Desmoplastic stroma	71,28	43,54		
	Mixed-type stroma	62,42	52,73		
Peritumoral lymphocytic infiltration	Absent	56,49	33,71	5,047 / -	**0,008***
	Present	81,96	44,34		
	Focal	57,44	34,10		
Intratumoral lymphocytic infiltration	Absent	57,27	32,01	- / -4,712	**0,000***
	Present	96,07	46,94		

Table 3. T test/ANOVA F test analysis of variables of recurrence-free survival and stromal reaction, intratumoral and peritumoral lymphocytic infiltration variables

	Variables	Average	Standard Deviation	F / t	p value
Stromal reaction	Absent	71,61	38,60	0,887 / -	0,451
	Fibromixoid stroma	49,89	28,74		
	Desmoplastic stroma	57,53	43,01		
	Mixed-type stroma	61,92	53,17		
Peritumoral lymphocytic infiltration	Absent	48,91	36,40	3,036 / -	**0,050***
	Present	70,30	44,85		
	Focal	52,44	36,26		
Intratumoral lymphocytic infiltration	Absent	51,69	34,05	- / -3,032	**0,003***
	Present	78,93	51,65		

DISCUSSION

The effect of immune system on cells going into a neoplastic process, is one of the important factors in tumor development. As the immune system initiates an inflammatory response in an effort to limit neoplastic progress, the immune cells in the tumor microenvironment becomes visible histologically and is thought to have an effect on progression-prognosis. Despite being proven to be important in several cancer types, tumoral lymphocytic reaction as a component of tumor microenvironment, is still a controversial a prognostic parameter in endometrial carcinomas (10).

Cancer Genome Atlas' study brings into question involving the molecular characteristics to tumor classification in order to eliminate inter-observer differences and evaluate biological mechanisms better and standardizing the treatment options. For this purpose, in accordance with the data obtained from the integrated results of somatic gene mutations, microsatellite instabilities and somatic copy number variations, endometrium cancers have been classified into four genomic groups. These groups include "POLE (polimerase epsilon) ultra-mutated", "MSI (microsatellite instability) hyper-mutated", "low copy number" and "high copy number". Tumors with POLE mutations are in the high-risk endometrial cancer group but don't show the expected poor prognostic progression despite their advanced stage. This is an important issue in treatment and follow-up of the patients. However, the high costs of molecular studies and the inability to find necessary equipment, prevent routine molecular grouping and studying the histopathological characteristics for differentiating these groups has become a necessity (11-15). A study by van Gool et al. reported a strong intratumoral lymphocytic (T lymphocytes) response in POLE proof reading-mutant cancers (8). Also, other studies in the literature are found to support this finding (14). These findings raise the question whether tumoral lymphocytic response would be considered a prognostic parameter.

It is possible to review tumoral lymphocytic reaction under two distinct groups; intratumoral lymphocytic reaction and peritumoral lymphocytic reaction. A study conducted by Workel et al. has demonstrated a relationship between the presence of intratumoral lymphocyte and prognosis in high-risk endometrial carcinoma cases (16). In another study, de Jong et al. reported that the presence of intratumoral lymphocytes is an independent prognostic factor in endometrial malignencies (17). In our study, intratumoral lymphocytic infiltration was observed in 28.57% of the cases. The overall survival period was 96,07±46,94 months in cases with intratumoral lymphocytic infiltration, and 57,27±32,01 months in cases without infiltration. Recurrence-free survival periods were 78,93±51,65 and 51,69±34,05 months, respectively.

In a study conducted by Kondratiev et al. on the relationship of intratumoral CD8+ T lymphocyte infiltration and survival in endometrial carcinomas, they reported lymphocytic infiltration at the margin of invasion to be an independent positive prognostic parameter[18]. In contrast, Ambros et al. found that peritumoral lymphocytic infiltration is not relevant in low grade endometrial carcinomas (19). In our study, the presence of peritumoral lymphocytic infiltration was observed in 45,92% of the cases. Peritumoral lymphocytic infiltration is significantly higher in tumors showing myometrial invasion less than half. Myometrial invasion depth is known to be a parameter indicated in the pathology reports and is closely related to metastasis and recurrence. This relationship between peritumoral lymphocytic infiltration and myometrial invasion depth supports the possibility of peritumoral lymphocytic reaction being a positive prognostic parameter in high-risk endometrioid carcinomas. In addition, when peritumoral lymphocytic infiltration was compared to survival status of the patients, a higher rate of peritumoral lymphocytic infiltration was detected in surviving group. When peritumoral reaction and overall and recurrence-free survival periods were compared, the survival periods were higher in cases showing peritumoral lymphocytic reaction. Overall and recurrence-free survival periods were 81,96±56,49 and 70,30±44,85 months, respectively, while it was 56,49±33,71 and 48,91±36,40 months in cases without infiltration. Therefore, we believe that this parameter should also take place in the pathology reports.

Although, stromal reaction has been evaluated in various cancers and determined to be in relation to poor clinical course, it has not yet been proven to be a prognostic parameter in endometrial cancers. In an endometrial carcinoma series including 400 cases, Panayiotou et al. evaluated the prognostic value of tumor stroma ratio and the relationship with other clinicopathological prognostic parameters, and determined low stromal reaction to be related to poor clinical course (20).

CONCLUSION

Presence of peritumoral and intratumoral lymphocytic infiltration positively affects prognosis, similar to results of other studies. These parameters are important and necessary in predicting the prognosis of the patients and they should be included in pathology reports.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Bulsa M, Urasińska E. Triple negative endometrial cancer. Ginekol Pol. 2017;88:212-4.
2. de Boer SM, Powell ME, Mileshkin L, Katsaros D, Bessette P, Haie-Meder C, et al. Adjuvant chemoradiotherapy versus radiotherapy alone for women with high-risk endometrial cancer (PORTEC-3): final results of an international, open-label, multicentre, randomised, phase 3 trial. Lancet Oncol. 2018;19:295-309
3. Keys HM, Roberts JA, Brunetto VL, Zaino RJ, Spirtos NM, Bloss JD, et al. A phase III trial of surgery with or without adjunctive external pelvic radiation therapy in intermediate risk endometrial adenocarcinoma: a Gynecologic Oncology Group study. Gynecol Oncol. 2004;92:744-51.
4. Uharcek P. Prognostic factors in endometrial carcinoma. J Obstet Gynaecol Res. 2008;34:776-83.
5. Singh N, Hirschowitz L, Zaino R, Alvarado-Cabrero I, Duggan MA, Ali-Fehmi R, at al. Pathologic Prognostic Factors in Endometrial Carcinoma (Other Than Tumor Type and Grade). Int J Gynecol Pathol. 2019;38:93-113.
6. Jakubowska K, Kisielewski W, Kańczuga-Koda L, Koda M, Famulski W. Oncol Lett. Stromal and intraepithelial tumor-infiltrating lymphocytes in colorectal carcinoma. 2017;14:6421-32.
7. Hendry S, Salgado R, Gevaert T, Russell PA, John T, Thapa B, et al. Assessing Tumor-Infiltrating Lymphocytes in Solid Tumors: A Practical Review for Pathologists and Proposal for a Standardized Method from the International Immuno-Oncology Biomarkers Working Group: Part 2: TILs in Melanoma, Gastrointestinal Tract Carcinomas, Non-Small Cell Lung Carcinoma and Mesothelioma, Endometrial and Ovarian Carcinomas, Squamous Cell Carcinoma of the Head and Neck, Genitourinary Carcinomas, and Primary Brain Tumors. Adv Anat Pathol. 2017;24:311-35.
8. van Gool IC, Eggink FA, Freeman-Mills L, Stelloo E, Marchi E, de Bruyn M, et al. POLE Proofreading Mutations Elicit an Antitumor Immune Response in Endometrial Cancer. Clin Cancer Res. 2015;21:3347-55.
9. Sofopoulos M, Fortis SP, Vaxevanis CK, Sotiriadou NN, Arnogiannaki N, Ardavanis A, et al. The prognostic significance of peritumoral tertiary lymphoid structures in breast cancer. Cancer Immunol Immunother. 2019;68:1733-45.
10. Hirata E, Sahai E. Tumor Microenvironment and Differential Responses to Therapy. Cold Spring Harb Perspect Med. 2017;7. pii: a026781.
11. Hoang LN, Kinloch MA, Leo JM, Grondin K, Lee CH, Ewanowich C, et al. Interobserver Agreement in Endometrial Carcinoma Histotype Diagnosis Varies Depending on The Cancer Genome Atlas (TCGA)-based Molecular Subgroup. Am J Surg Pathol. 2017;41:245-52.
12. Talhouk A, McAlpine JN. New classification of endometrial cancers: the development and potential applications of genomic-based classification in research and clinical care. Gynecol Oncol Res Pract. 2016;3:14.
13. Church DN, Stelloo E, Nout RA, Valtcheva N, Depreeuw J, ter Haar N, et al. Prognostic significance of POLE proofreading mutations in endometrial cancer. J Natl Cancer Inst. 2014;107:402.
14. Hussein YR, Weigelt B, Levine DA, Schoolmeester JK, Dao LN, Balzer BL, et al. Clinicopathological analysis of endometrial carcinomas harboring somatic POLE exonuclease domain mutations. Mod Pathol. 2015;28:505-14.
15. Billingsley CC, Cohn DE, Mutch DG, Hade EM, Goodfellow PJ. Prognostic Significance of POLE Exonuclease Domain Mutations in High-Grade Endometrioid Endometrial Cancer on Survival and Recurrence: A Subanalysis. Int J Gynecol Cancer. 2016;26:933-8.
16. Workel HH, Komdeur FL, Wouters MC, Plat A, Klip HG, Eggink FA, et al. CD103 defines intraepithelial CD8+ PD1+ tumour-infiltrating lymphocytes of prognostic significance in endometrial adenocarcinoma. Eur J Cancer. 2016;60:1-11.
17. de Jong RA, Leffers N, Boezen HM, ten Hoor KA, van der Zee AG, Hollema Het al. Presence of tumor-infiltrating lymphocytes is an independent prognostic factor in type I and II endometrial cancer. Gynecol Oncol. 2009;114:105-10.
18. Kondratiev S, Sabo E, Yakirevich E, Lavie O, Resnick MB. Intratumoral CD8+ T lymphocytes as a prognostic factor of survival in endometrial carcinoma. Clin Cancer Res. 2004;10:4450-6.
19. Ambros RA, Kurman RJ Combined assessment of vascular invasion and myometrial invasion as a model to predict prognosis in stage I endometrioid adenocarcinoma of uterine corpus. Cancer. 1992,69:1424-31.
20. Panayiotou H, Orsi NM, Thygesen HH, Wright AI, Winder M, Hutson R, et al. The prognostic significance of tumour-stroma ratio in endometrial carcinoma. BMC Cancer. 2015;15:955.