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Immunohistochemistry Staining for the Mismatch Repair Proteins in Endometrial Cancer Patients

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ABSTRACT

Objectives: Lynch syndrome (LS) increases the lifetime risks of endometrial cancer by approximately 40-60%. Although universal screening with immunohistochemistry (IHC) for mismatch repair (MMR) proteins has been recommended, it is not yet common in Thailand. This study aimed to evaluate the prevalence of MMR deficiency and identify patients who may be at risk for LS.

Materials and Methods: IHC for MMR proteins, including mutL homolog 1 (MLH1), mutS homolog 2 (MSH2), mutS homolog 6 (MSH6), and PMS1 homolog 2 (PMS2), were tested in 156 endometrial cancer patients who underwent primary surgery between 2013-2015. This study also screened for the revised Bethesda guidelines, using age at diagnosis and personal and family history of LS-related cancers as variables.

Results: Fifty-seven of 156 (35.9%) patients had MMR deficiency; 42 experienced losses of MLH1 and PMS2, 10 experienced losses of MSH2 and MSH6, and 5 experienced a loss of MSH6 expression. Only 36 patients (23.1%) met the revised Bethesda guidelines; 29 patients (18.6%) were diagnosed earlier than age 50; 10 patients (6.4%) had synchronous colon or ovarian cancer; and only 13 patients (8.3%) possessed a family history of LS-related cancers. It was possible to detect MMR deficiency in 41/120 patients (34.2%) who did not meet the revised Bethesda guidelines.

Conclusion: MMR deficiency as a result of IHC can be detected in 35.9% of endometrial cancer patients. However, it was still possible to detect MMR deficiency in at least one-third of patients who did not meet the Bethesda guidelines. Screening endometrial cancer patients for MMR IHC should be considered, with the aim of diagnosing and preventing LS-related cancers in both patients and their relatives.

Keywords: endometrial cancer, lynch syndrome, hereditary nonpolyposis colorectal cancer, immunohistochemistry, mismatch repair proteins.

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การย้อมอิมมูโนพยาธิวิทยาโปรตีนมิสแมชรีแพร์ในผู้ป่วยมะเร็งเยื่อบุโพรงมดลูก

พิมพ์พิชชา พวงศรีเจริญ, ธาริณี แม่นชนะ, ชัย อริยศรีวัฒนา, สุรางค์ ตรีรัตนชาติ

บทคัดย่อ

วัตถุประสงค์: *ลินช์ซินโดรม (Lynch Syndrome) เพิ่มความเสี่ยงต่อการเป็นมะเร็งเยื่อบุโพรงมดลูก 40-60% ในต่างประเทศโดยเฉพาะในองค์กรณ์ใหญ่ๆ แนะนำให้ตรวจด้วยเทคนิคทางอิมมูโนพยาธิวิทยาโปรตีนมิสแมชรีแพร์ในผู้ป่วยมะเร็งลำไส้หรือมะเร็งเยื่อบุโพรงมดลูก อย่างไรก็ตามในประเทศไทยยังไม่ได้มีการนำเทคนิคการย้อมอิมมูโนพยาธิวิทยาโปรตีนมิสแมชรีแพร์มาใช้แพร่หลายในผู้ป่วย งานวิจัยนี้ทำขึ้นเพื่อศึกษาอัตราการย้อมไม่ติดของชิ้นเนื้อด้วยเทคนิคทางอิมมูโนพยาธิวิทยาดังกล่าวในผู้ป่วยมะเร็งเยื่อบุโพรงมดลูกในประเทศไทย*

วัสดุและวิธีการ: *ศึกษาในผู้ป่วยมะเร็งเยื่อบุโพรงมดลูกที่ทำการผ่าตัดที่โรงพยาบาลจุฬาลงกรณ์ ระหว่างปี 2556-2558 รวมทั้งสิ้น 156 ราย นำบล็อกชิ้นเนื้อเยื่อบุโพรงมดลูกตัดลงสไลด์ และทำการย้อมชิ้นเนื้อด้วยเทคนิคทางอิมมูโนพยาธิวิทยาโปรตีนมิสแมชรีแพร์ (mismatch repair) ได้แก่ MLH1, MSH2, MSH6, PMS2 อ่านผลอัตราการย้อมไม่ติดสีโดยพยาธิแพทย์ทางนรีเวช 2 ท่าน รวมถึงคัดกรองประวัติตาม Revised Bethesda guideline ได้แก่ อายุที่ได้รับการวินิจฉัย ประวัติมะเร็งของผู้ป่วยและครอบครัว จากเวชระเบียนและจากผู้ป่วย*

ผลการศึกษา: *ตรวจพบอัตราการย้อมไม่ติดอิมมูโนพยาธิวิทยาของโปรตีนมิสแมชรีแพร์ทั้งสิ้น 57 ราย จากผู้ป่วย 156 ราย (35.9%) แยกเป็นย้อมไม่ติดของ MLH1/PMS2 42 ราย MSH2/MSH6 10 ราย และ MSH6 5 ราย การคัดกรองโดยใช้ Revised Bethesda guideline พบว่ามีผู้ป่วยที่เข้าได้ตามเกณฑ์ทั้งสิ้น 36 ราย (23.1%) โดยพบผู้ป่วยที่ได้รับวินิจฉัยเป็นมะเร็งก่อนอายุ 50 ปี จำนวน 29 ราย (18.6%) ผู้ป่วย 10 ราย (6.4%) พบมะเร็งลำไส้หรือมะเร็งรังไข่ร่วมกับมะเร็งเยื่อบุโพรงมดลูก ผู้ป่วย 13 ราย (8.3%) มีประวัติครอบครัวเข้าได้กับ Lynch syndrome อย่างไรก็ตามผู้ป่วย 120 ราย ที่ประวัติไม่ตรงตามเกณฑ์ Revised Bethesda guideline ตรวจพบการย้อมไม่ติดอิมมูโนพยาธิวิทยาของโปรตีนมิสแมชรีแพร์ 41 ราย คิดเป็น 34.2%*

สรุป: *อัตราการย้อมไม่ติดของยีนในกลุ่มมิสแมชรีแพร์ในผู้ป่วยมะเร็งเยื่อบุโพรงมดลูกคิดเป็น 35.9% ในผู้ป่วยที่ไม่พบประวัติความเสี่ยงที่เข้าได้กับ Bethesda guideline สามารถตรวจพบการย้อมไม่ติดของยีนในกลุ่มมิสแมชรีแพร์ได้ถึง 1 ใน 3 ดังนั้นการตรวจคัดกรองทางอิมมูโนพยาธิวิทยาควรพิจารณาทำในผู้ป่วยมะเร็งเยื่อบุโพรงมดลูกทุกราย ผู้ป่วยรายนั้นจะได้รับการปรึกษาทางพันธุกรรม (genetic counseling) เพื่อรับการตรวจทางพันธุกรรมต่อไป รวมถึงให้คำปรึกษากับญาติผู้ป่วยที่มีความเสี่ยง*

คำสำคัญ: *มะเร็งเยื่อบุโพรงมดลูก, อิมมูโนพยาธิวิทยาโปรตีนมิสแมชรีแพร์, ลินช์ซินโดรม*

Introduction

Endometrial cancer is the most common gynecologic malignancy in developed countries[1]. In Thailand, it is the third most common gynecologic malignancy, following cervical cancer and ovarian cancer, which appear in 13.4% and 4.4% of the population, respectively[2]. Approximately 3-5% of endometrial cancer can be attributed to Lynch syndrome (LS), previously known as hereditary nonpolyposis colorectal cancer (HNPCC)[1]. This syndrome is an autosomal dominant disease caused by germline mutations of mismatch repair (MMR) genes (mutL homolog 1 (MLH1), mutS homolog 2 (MSH2), mutS homolog 6 (MSH6), PMS1 homolog 2 (PMS2), and epithelial cell adhesion molecule (EPCAM)). LS patients have a 40-60% lifetime risk of getting endometrial cancer and colon cancer[1, 3].

In general, personal and family history of cancer is used as a screening tool. The Amsterdam criteria were developed in 1990 and have subsequently been modified and become the Amsterdam II criteria. These criteria exhibit high specificity, but low sensitivity. In 1997 the Bethesda guidelines were developed; They were then revised in 2004. In contrast to the Amsterdam criteria, the Bethesda guidelines possess a high degree of sensitivity[1]. However, screening LS using both criteria will still result in a misdiagnosis of at least 30% of patients[4].

Molecular tumour testing, such as immunohistochemistry (IHC) for MMR genes expression, microsatellite instability testing, and MLH1 promoter methylation testing, have been endorsed for universal screening use in all endometrial cancer patients[1, 5]. However, IHC for MLH1, MSH2, MSH6, and PMS2 expression is the most cost-effective, and it is widely available in most pathology laboratories[6]. This screening test is not yet recommended in Thailand, however. In addition, In Thailand, there is no published data about the prevalence of MMR deficiency in endometrial cancer patients. This study may be the first study in Thailand that aimed to identify endometrial cancer patients at risk of LS.

Materials and Methods

Immunohistochemistry screening with MMR proteins was performed in endometrial cancer patients who had undergone primary surgery at King Chulalongkorn Memorial Hospital in Bangkok, Thailand between January 2013 and December 2015. Demographic data, such as age at diagnosis, parity, menopausal status, body mass index (BMI), family or personal history of cancer, pathological data, and received treatment, were retrieved from the medical records. Endometrial cancer patients who met the revised Bethesda guidelines were as follows; less than 50 years old at time of diagnosis; synchronous or metachronous ovarian, colon or other LS-related cancer at any age; first degree relative with LS-related cancer who was diagnosed before 50 years of age or two or more relatives with LS-related cancer at any age[7]. This study was approved by the Institutional Review Board, Faculty of Medicine, at Chulalongkorn University (IRB015/60).

Immunohistochemistry for MMR proteins includes MLH1, MSH2, MSH6, and PMS2. Leica RM2245, a semi-motorized rotary microtome, was used to place a 5-mm-thick, formalin-fixed, paraffin-embedded section onto a 2-micron tissue slide. Primary monoclonal antibodies against MLH1 (clone G168-15; Zytomed system; Berlin, Germany), MSH2 (clone FEE11; Zytomed system; Berlin, Germany), MSH6 (clone SPM525; Zytomed System; Berlin, Germany), and PMS2 (clone EPR3947; Zytomed system; Berlin, Germany) were applied to 2-micron tissue slides. Antigen retrieval was performed using Dako autostainer Link48's proprietary antigen retrieval solution at pH 8.0 (MLH1, MSH2, MSH6, and PMS2). All slides were reviewed by two pathologists. Normal expression is defined as nuclear staining within tumour cells, using the nuclei of infiltrating lymphocytes and/or normal stromal cells as positive internal control. Negative expression is defined as the complete absence of nuclear staining within tumour cells, but the presence of staining in normal endometrial and stromal cells. MMR deficiency is defined as the negative or loss expression of at least one of the four MMR proteins.

(Fig.1). Mutual agreement had been made between two pathologists.

A statistical analysis was conducted using SPSS version 22.0 (IBM Corp., Armonk, N.Y., USA). Categorical variables were calculated using the chi-square or Fisher exact tests. Continuous variables were tested using the student t-test. Statistical significance was achieved when the p value was less than 0.05.

Results

A total of 156 endometrial cancer patients were included. Demographic data and pathological data are presented in Table 1. The mean age is 57.1 ± 11.0 years (range 20-83 years). Most patients (73.7%) were in stage I. Stage II, III, and IV were account for 7.1%, 17.3%, and 1.9% of patients, respectively. Thirty-six patients (23.1%) met the revised Bethesda guidelines; 29 patients (18.6%) were diagnosed at an age of less than 50 years; 13 patients (8.3%) had a family history of LS related-cancer (2 patients had first degree relative with LS-related cancer diagnosed before 50 years and the remaining 11 patients had two or more relatives with LS-related cancer at any age); and 10 patients (6.4%) had synchronous endometrial and ovarian or colon cancers.

Fifty-seven of 156 patients (35.9%) had an MMR deficiency; 42 experienced a loss of MLH1 and PMS2 (26.9%); 10 experienced a loss of MSH2 and MSH6 (6.4%); and 5 experienced a loss of MSH6 expression (3.2%). There was no significant difference in the clinicopathological characteristics between patients with and without MMR deficiency, except family history of LS-related cancers. More patients in the MMR deficient group possessed a family history of LS-related cancers: 15.8% and 4%, respectively (p=0.02). Sixteen of the 36 patients (44.4%) who met the revised Bethesda guidelines exhibited a loss of MMR expression. In contrast, MMR deficiency was still detected in 41 of 120 patients (34.2%) who did not meet the revised Bethesda guidelines. MMR deficiency was detected in less than half of the patients when the following criteria were used: younger than 50 years old when diagnosed and presence of synchronous cancers; 44.8% (13/29 patients) and 40% (4/10 patients), respectively.

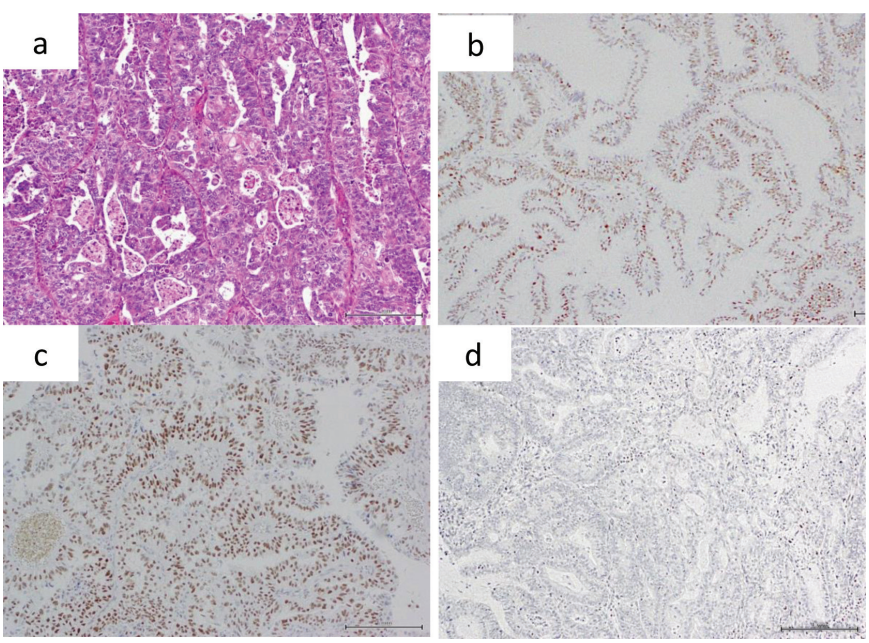

Fig. 1. H&E slide on tumour cells. (a) Tumour cells express nuclear MLH1 (b) and PMS2. (c) Tumour cells do not express MSH6. (d)

Table 1. Demographic data and pathological findings.

	All cases (N=156)	MMR deficiency (N=57)	MMR proficiency (N=99)	p value
Age, mean ± SD (years)	57.1 ± 11.0	56.0 ± 11.0	57.7 ± 11.1	0.35
Age ≤ 50 years	29 (18.6%)	13 (22.8%)	16 (16.2%)	0.39
Nulliparous	62 (39.7%)	21 (36.8%)	41 (41.4%)	0.61
Menopause	103 (66.0%)	35 (61.4%)	68 (68.7%)	0.38
BMI, mean ± SD (kg/m^2)	26.9 ± 6.6	27.6 ± 6.5	26.5 ± 6.7	0.31
Family history of cancers	13 (8.3%)	9 (15.8%)	4 (4.0%)	0.02
Bethesda guidelines	36 (23.1%)	16 (28.1%)	20 (20.2%)	0.32
Stage				0.65
I	115 (73.7%)	45 (78.9%)	70 (70.7%)	
II	11 (7.1%)	4 ((7.0%)	7 (7.1%)	
III	27 (17.3%)	7 (12.3%)	20 (20.2%)	
IV	3 (1.9%)	1 (1.8%)	2 (2.0%)	
Histology				0.41
Endometrioid carcinoma	149 (95.5%)	55 (96.5%)	94 (94.9%)	
Mixed adenocarcinoma	5 (3.2%)	1 (1.8%)	4 (4.0%)	
Papillary serous carcinoma	1 (0.6%)	1 (1.8%)	0 (0%)	
Carcinosarcoma	1 (0.6%)	0 (0%)	1 (1.0%)	
Tumor grade				0.14
G1	86 (55.1%)	28 (49.1%)	58 (58.6%)	
G2	32 (20.5%)	10 (17.5%)	22 (22.2%)	
G3	38 (24.4%)	19 (33.3%)	19 (19.2%)	
Myometrial invasion				0.49
< 50%	97 (62.2%)	33 (57.9%)	64 (64.6%)	
≥ 50%	59 (37.8%)	24 (42.1%)	35 (35.4%)	
LVSI (N=100)	43 (43.0%)	17/35 (48.6%)	26/65 (40.0%)	0.62
Lower uterine segment involvement	100 (64.1%)	37 (64.9%)	63 (65.6%)	1.00
Pelvic node metastasis (N=141)	16 (11.3%)	4/53 (7.5%)	12/88 (13.6%)	0.38
Paraaortic node metastasis (N=80)	5 (6.3%)	2/33 (6.1%)	3/47 (6.4%)	0.46
Synchronous endometrial and ovarian and/or colon cancers	10 (6.4%)	4 (7.0%)	6 (6.1%)	1.00
Adjuvant treatment				0.46
None	64 (41.0%)	19 (33.3%)	45 (45.5%)	
Pelvic radiation with brachytherapy	28 (17.9%)	13 (22.8%)	15 (15.2%)	
Concurrent chemoradiation	24 (15.4%)	8 (14.0%)	16 (16.2%)	
Brachytherapy	21 (13.5%)	10 (17.5%)	11 (11.1%)	
Pelvic radiation then adjuvant chemotherapy	1 (0.6%)	1 (1.8%)	0 (0%)	
Chemotherapy then pelvic radiation	4 (2.6%)	1 (1.8%)	3 (3.0%)	
Chemotherapy	14 (9.0%)	5 (8.8%)	9 (9.1%)	

MMR: mismatch repair, SD: standard deviation, LVSI: lymphovascular invasion.

Discussion

Universal screening with IHC for MMR proteins in endometrial cancer is recommended in many countries. The loss rate of MMR IHC expression was reported between 24-34%[8-12]. Our study demonstrated a loss rate of 35.9%. This finding is consistent with Spanish studies, which found approximately 34%[8]. Studies from USA (20-25%)[9,12] and China (24%)[10], reported lower rates of MMR deficiency. Thus, different ethnicities might influence the findings. The MLH1/PMS2 proteins exhibited loss of expression most often. Our study reported approximately 73.6% loss of expression; this rate is similar to previous studies that reported between 72-81%[9,12]. The revised Bethesda guidelines remain the current clinical criteria for the identification of endometrial patients at risk of having LS in Thailand. In our study, MMR deficiency was detected in one-third of the patients who did not meet the Bethesda guidelines. Recent published data indicates that approximately 41% of patients can be undiagnosed using traditional LS screening[13]. There is much evidence to support universal screening for LS with microsatellite instability (MSI) and/or IHC for MMR proteins in all colorectal carcinoma[7,14]. It appears that MSI is less sensitive than IHC in detecting MSH6 mutation carriers, which exhibit a higher lifetime risk of developing endometrial cancer than colorectal cancer[4]. Although there is evidence to confirm that both MSI and IHC possess excellent sensitivity and specificity for identifying patients with LS, IHC is sufficient for determining MMR deficiency in endometrial cancer. The concordant rate between these two techniques was approximately 94-100%[15-17]. However, IHC is more advantageous than MSI in many aspects: it is less expensive, it is widely available in most pathological centres, and it can guide specific MMR genes that are most likely to have a germline mutation. Nevertheless, improper tissue fixation can result in weak or equivocal staining patterns or be less reliable[14].

Identification of MMR deficient status is beneficial for several reasons. First, an assessment of the molecular classification in comparison to pathological risk groups can be used as a prognostic factor, especially in early stage endometrial cancer. Stage 1 endometrial cancer patients with intermediate to high risk factors, combined with MMR deficiency, exhibited a higher recurrence rate than those with MMR proficiency. It may reduce over-treatment in favourable cases and select unfavourable cases for more intensive treatment[18,19]. Second, it can guide adjuvant treatment, as patients with MMR deficiency may respond to immunotherapy[17]. Third, further genetic testing should be offered to confirm LS. This information may be helpful for guiding further investigation and treatment. LS increases the risk of synchronous and metachronous malignancies compared with the general population. It has been well established that endometrial cancer often precedes colorectal and other LS-related malignancies. In more than half of the patients, gynecologic cancer occurred before the diagnosis of other LS-related cancers with a median duration of 11 years[20]. Therefore, comprehensive cancer surveillance and risk-reducing surgeries should be considered. If LS was established at the time of endometrial biopsy, this information may influence the treatment options, especially in young women. There is a trend of increasing the incidence of endometrial cancer in the young. In this study, 19% of patients were younger than 50 years and 10% were younger than 40 years[21]. Conservative medical treatments or fertility sparing surgery to conserve ovaries should be discussed. Patients should also realize and weigh the risks of synchronous ovarian cancer and worsening prognosis by delaying surgery[13]. Because of its hereditary basis, a genetics evaluation should be offered to patients' family members to identify those who may be at risk and recommend LS-related cancer surveillance.

MMR deficiency by IHC was detected in 35.9% of endometrial cancer patients in our study. Tumour testing with IHC is inexpensive and available in most pathology laboratories. If expression of an MMR protein is absent in any gene, the patients should be offered genetic counselling and further genetic testing. If all four MMR proteins are expressed, the presence of LS is unlikely. Further study to confirm germline MMR mutation in patients with MMR deficiency is ongoing. This study might be the first study to evaluate the

incidence of LS in Thai patients with endometrial cancer. MMR deficiency was still detected in at least one-third who did not meet the revised Bethesda guidelines. Screening endometrial cancer patients for MMR IHC should be considered to diagnose and prevent LS-related cancers in both patients and their relatives.

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Potential conflicts of interest

The authors declare no conflict of interest.

References

1. ACOG Practice Bulletin No. 147: Lynch syndrome. Obstet Gynecol 2014;124:1042-54.
2. GLOBOCAN. Estimated incidence, mortality and 5-year prevalence: women 2012 [Available from: http://globocan.iarc.fr/Pages/fact_sheets_population.aspx.
3. Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. Clin Genet 2009;76:1-18.
4. Hampel H, Frankel W, Panescu J, Lockman J, Sotamaa K, Fix D, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res 2006;66:7810-7.
5. Lancaster JM, Powell CB, Chen LM, Richardson DL. Society of Gynecologic Oncology statement on risk assessment for inherited gynecologic cancer predispositions. Gynecol Oncol 2015;136:3-7.
6. Barrow E, Hill J, Evans DG. Cancer risk in Lynch Syndrome. Fam Cancer 2013;12:229-40.
7. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004;96:261-8.
8. Egoavil C, Alenda C, Castillejo A, Paya A, Peiro G, Sanchez-Heras AB, et al. Prevalence of Lynch syndrome among patients with newly diagnosed endometrial cancers. PLoS One 2013;8:e79737.
9. Moline J, Mahdi H, Yang B, Biscotti C, Roma AA, Heald B, et al. Implementation of tumor testing for lynch syndrome in endometrial cancers at a large academic medical center. Gynecol Oncol 2013;130:121-6.
10. Long Q, Peng Y, Tang Z, Wu C. Role of endometrial cancer abnormal MMR protein in screening Lynch-syndrome families. Int J Clin Exp Pathol 2014;7:7297-303.
11. Rubio I, Ibanez-Feijoo E, Andres L, Aguirre E, Balmana J, Blay P, et al. Analysis of Lynch Syndrome Mismatch Repair Genes in Women with Endometrial Cancer. Oncology 2016;91:171-6.
12. Watkins JC, Yang EJ, Muto MG, Feltmate CM, Berkowitz RS, Horowitz NS, et al. Universal Screening for Mismatch-Repair Deficiency in Endometrial Cancers to Identify Patients With Lynch Syndrome and Lynch-like Syndrome. Int J Gynecol Pathol 2017;36:115-27.
13. Mills AM, Liou S, Ford JM, Berek JS, Pai RK, Longacre TA. Lynch syndrome screening should be considered for all patients with newly diagnosed endometrial cancer. Am J Surg Pathol 2014;38:1501-9.
14. Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. J Mol Diagn 2008;10:293-300.
15. Walsh M, Cummings M, Buchanan D, DambacherW, Arnold S, McKeone D, et al. Molecular, pathologic, and clinical features of early-onset endometrial cancer: identifying presumptive Lynch syndrome patients. Clin Cancer Res 2008;4:1692-700.
16. Leenen CH, van Lier MG, van Doorn HC, van Leerdam ME, Kooi SG, de Waard J, et al. Prospective evaluation of molecular screening for Lynch syndrome in patients with endometrial cancer ≤ 70 years. Gynecol Oncol 2012;125:414-20.
17. Stelloo E, Jansen AML, Osse EM, Nout RA, Creutzberg CL, Ruano D, et al. Practical guidance for mismatch repair-deficiency testing in endometrial cancer. Ann Oncol 2017;28:96-102.
18. Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, et al. A clinically applicable molecular-based classification for endometrial cancers. Br J Cancer 2015;113:299-310.
19. Stelloo E, Nout RA, Osse EM, Jurgenliemk-Schulz IJ, Jobsen JJ, Lutgens LC, et al. Improved Risk Assessment by Integrating Molecular and Clinicopathological Factors in Early-stage Endometrial Cancer-Combined Analysis of the PORTEC Cohorts. Clin Cancer Res 2016;22:4215-24.
20. Lu KH, Dinh M, Kohlmann W, Watson P, Green J, Syngal S, et al. Gynecologic cancer as a "sentinel cancer" for women with hereditary nonpolyposis colorectal cancer syndrome. Obstet Gynecol 2005;105:569-74.
21. Manchana T, Khemapech N. Endometrial adenocarcinoma in young Thai women. Asian Pac J Cancer Prev 2008;9:283-6.