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Clinical significance of mismatch repair genes immunohistochemical expression of complex endometrial hyperplasia

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Objective
Women with Lynch syndrome have an increased risk of developing colorectal and gynecologic malignancies such as endometrial cancer. Complex hyperplasia has about a 30% risk of developing into endometrial cancer. The aim of this study was to determine the genetic risk for developing endometrial cancer by immunohistochemical staining of premalignant lesions for mutL homolog 1, mutS homolog 2, mutS homolog 6, and postmeiotic segregation increased 2.

Methods
Twenty cases (n=20) were selected from among patients with available sample blocks for analysis. Clinical information was obtained from medical chart review. Immunohistochemical staining was performed for all of the tumor blocks. Staining was scored based on the intensity (intensity score 0–3) .

Results
Among the 20 cases of complex endometrial hyperplasia, 11 (55%) patients showed loss of expression of at least one of the following proteins: mutL homolog 1, mutS homolog 2, mutS homolog 6, or postmeiotic segregation increased 2. Seven (35%) patients were negative for the expression of two or more proteins, and one patient (5%) was negative for the expression of all four proteins.

Conclusion
More than half of the patients showed loss of expression of at least one mismatch repair protein in our study population. Genetic risk counseling and further tests are recommended for these patients.

Keywords: Endometrial hyperplasia; Endometrial premalignancy; Mismatch repair gene

Introduction

Endometrial carcinoma is the second most frequent gynecologic malignancy in women with 49,560 cases reported and 8,190 deaths from this disease in the United States in 2013 [1]. Endometrial carcinoma typically arises from atypical complex hyperplasia and develops in estrogen-rich environments, such as those that are present in the cases of obesity, anovulation, and excessive exogenous estrogen [2]. The endometrioid subtype of endometrial adenocarcinoma comprises 80% to 85% of cancers arising from the lining of the endometrium and is frequently preceded by a precursor lesion, such as endometrial hyperplasia [3]. The cumulative 20-year progression risk among women who remain at risk for at least 1 year is 28% for atypical complex hyperplasia; this is 14-fold the risk faced by women with non-atypical endometrial hyperplasia [4].

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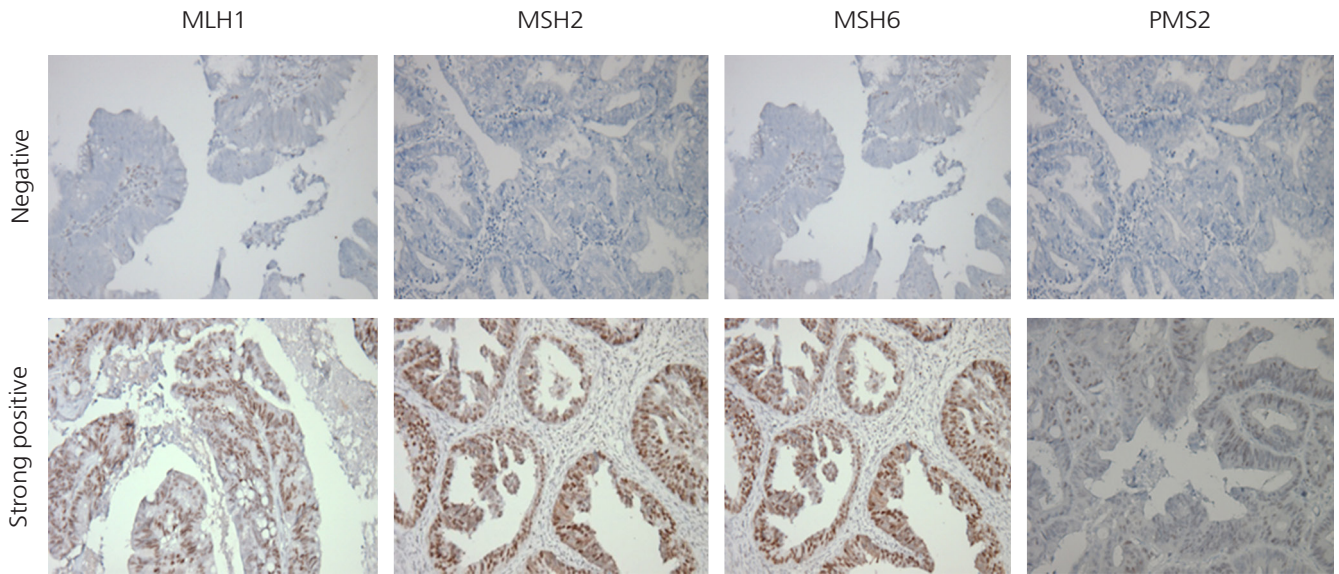|	MLH1	MSH2	MSH6	PMS2

Fig. 1. Immunohistochemistry examples of the comparative expression of mutL homolog 1 (MLH1), mutS homolog 2 (MSH2), mutS homolog 6 (MSH6), and post-meiotic segregation increased 2 (PMS2) in complex endometrial hyperplasia lesion (PMS2 picture show only mild positive expression).

The detection and appropriate treatment of complex endometrial hyperplasia with atypia are important to prevent endometrial carcinoma. About 5% of endometrial carcinoma cases are thought to be caused by inherited genetic changes [5]. Lynch syndrome or hereditary nonpolyposis colorectal cancer (HNPCC) accounts for 2% to 3% of all endometrial carcinomas [6]. Lynch syndrome is an autosomal dominant disorder known to occur at various extracolonic sites [7].

Most Lynch syndrome cases are caused by germline mutations of DNA mismatch repair genes (mutL homolog 1 [MLH1], mutS homolog 2 [MSH2], mutS homolog 6 [MSH6], and post-meiotic segregation increased 2 [PMS2]) [8-13]. These genetic defects in the DNA mismatch repair system result in replication errors in repetitive DNA segments, known as microsatellite instability (MSI), and the absence of expression of mismatch repair proteins in the tumor. Currently, Lynch syndrome is diagnosed based on family history, immunohistochemistry (IHC), MSI, and gene sequencing chromatograms (revised Amsterdam, 1998 [7] or Bethesda, 2002 [14] criteria).

More than 75% of endometrial tumors in HNPCC mutation carriers show MSI [15,16]. Furthermore, HNPCC-related endometrial cancer studies have shown a correlation between MSI and the lack of related protein expression as detected by IHC [17-20].

Our aim was to investigate the association between premalignant lesions of the endometrium by evaluating MLH1, MSH2, MSH6, and PMS2 expression in patients with complex endometrial hyperplasia by IHC to find appropriate group for further genetic counseling and test.

Materials and methods

1. Patient characteristics
The study sample was identified through a retrospective search of medical records. Among the patients with pathologically proven complex endometrial hyperplasia who were treated at the Department of Obstetrics and Gynecology, Samsung Changwon Hospital from 2001 to 2012, 20 patients with available blocks were chosen. Their medical records were reviewed for clinical characteristics. After acquiring approval from the institutional review board (IRB) at the Samsung Changwon Hospital (IRB no. 2012-SCMC-028-00), IHC staining of MSH2, MLH1, MSH6, and PMS2 was performed.

After confirmation of the pathologic diagnosis of complex endometrial hyperplasia, the 20 patients were treated with either medical therapy (e.g., progesterone) or surgical treatment such as hysterectomy.

2. Immunohistochemical staining
IHC analysis of endometrial hyperplasia specimens was performed to determine the protein expression of MLH1 (Novocas-

tra, Newcastle, UK), MSH2 (Novocastra), MSH6 (Novocastra), and PMS2 (Novocastra). Immunohistochemical staining was performed using the streptavidin-biotin method. Sections (4-μm thick) were deparaffinized with xylene, rehydrated, and incubated with fresh 0.3% hydrogen peroxide in methanol for 30 min at room temperature. Specimens were rehydrated through a graded ethanol series and washed in phosphate-buffered saline (PBS). After blocking treatment, specimens were incubated with monoclonal antibodies at a dilution of 1:200 in PBS containing 1% bovine serum albumin at 4°C overnight. Sections were washed with PBS and incubated in secondary antibody for 30 minutes at room temperature. The chromogen was a 3.3% to 0.02% solution containing 0.005% H_2O_2 in 50 mM ammonium acetate-citric acid buffer (pH 6.0). Tissue sections were lightly counterstained with hematoxylin and then examined by light microscopy. There was no detectable staining in the negative controls prepared by omitting the primary antibody.

3. Evaluation

Staining was evaluated by two independent observers without knowledge of clinical outcomes. One dedicated gynecologic pathologist and one gynecologic oncologist reviewed the slides in a blinded manner and evaluated the immunohistochemical data independently. The staining intensity was scored as follows: 0, no appreciable staining in tumor cells; 1, barely detectable staining in the cytoplasm, nucleus, or membrane compared with stromal elements; 2, readily detectable brown staining distinctly marking the tumor cell cytoplasm, nucleus, or membrane; and 3, dark brown staining in tumor cells completely obscuring the cytoplasm, nucleus, or membrane (Fig. 1). A previous study regarding Lynch syndrome reported that complete loss of expression of these genes in the setting of a positive internal control is interpreted as a positive result [21].

Results

The demographic and clinical characteristics of the 20 patients are described in Table 1. The median age at diagnosis was 49 years (range, 38 to 66 years). The median body mass index of the patients was 24.19, and 35% of the patients had a body mass index above 25. Parity was known in all 20 patients. Of these, two (10%) were nulliparous.

After the dilatation and curettage biopsy (DCB) diagnosis of complex hyperplasia, four (20%) patients received medical

Table 1. Demographic and clinical characteristics

Characteristic	Value
Median age (yr)	49 (38–66)
Premenopausal	11 (55)
Body mass index	24.2 (19.3–34.6)
Nulliparous	2 (10)
Family history of cancer	None
Treatment type	
Medication (progestin therapy)	4 (20)
Hysterectomy after medical treatment	2 (10)
Hysterectomy only	11 (55)
Follow-up without treatment	3 (15)
Operation type	
DCB only	7 (35)
Hysterectomy after DCB	12 (60)
Hysterectomy only	1 (5)
Final Pathology	
Complex hyperplasia without atypia	4 (20)
Complex hyperplasia with atypia	14 (70)
Endometrial cancer	2[a] (10)

Values are presented as n (range) or n (%).
DCB, dilatation and curettage biopsy.
[a]Two patients were complex hyperplasia with atypia at DCB but endometrial cancer (endometrioid type grade I) was diagnosed at hysterectomy.

treatment (progesterone) for 3 months only, and the follow-up endometrial biopsy revealed no lesion, two (10%) patients received medical treatment and surgical treatment (hysterectomy) with persistent disease, and 11 (55%) underwent surgical treatment only. Three (15%) patients refused treatment after diagnosis (Table 1). Among the surgery types, DCB only was performed in seven patients, hysterectomy after DCB in 12 patients, and hysterectomy only in one patient. The mean medication treatment period for six patients was 24 months. Of the 20 cases of complex hyperplasia, four cases were diagnosed with complex hyperplasia without atypia, and 16 (80%) with complex hyperplasia with atypia after the final pathology.

After surgery as an adjuvant treatment, endometrial cancer was found incidentally by pathological analysis in two of the 16 patients diagnosed with complex hyperplasia with atypia. Surgical staging was performed for these two patients; both had endometrial cancer stage 1, and no additional treatment was applied.

The results of the IHC expression of MLH1, MSH2, MSH6, and PMS2 are summarized in Table 2. Eleven patients (55%) showed loss of expression of at least one of the following proteins: MLH1,

MSH2, MSH6, or PMS2. Seven patients (35%) were negative for the expression of two or more proteins, and one patient (5%) was negative for the expression of all four proteins (Table 2).

Discussion

Approximately 247,000 and 1,600,000 new cancer cases are expected to occur in Korea and the United States [1] in 2013, respectively. In Korea, the expected incidence and mortality due to endometrial cancer are expected to be 2,175 and 262 cases, respectively [22]. Appropriate cancer treatment and early diagnosis and prevention are required to decrease mortality and the economic burden associated with cancer diagnosis.

The present study is the first to determine the correlation between mismatch repair gene (MLH1, MSH2, MSH6, and PMS2) protein expression and complex hyperplasia using IHC in a Korean population. Further genetic counseling, tests, and long-term follow up are needed to prevent endometrial carcinoma in this subgroup of patients.

IHC is an easy and inexpensive test. The sensitivity and specificity of IHC for predicting Lynch syndrome are both near 100% [23]. Complex hyperplasia has a high cumulative progression risk for endometrial carcinoma. The identification of patients with a genetic risk among those with complex hyperplasia based on IHC is important for cancer prevention and early detection. However, IHC is not a confirmative test, and gene sequencing tests are expensive. Furthermore, the evaluation of IHC can vary among pathologists.

A previous study showed that loss of MLH1 expression was correlated with the loss of PMS2 and that the loss of MSH2 expression was associated with the loss of MSH6 expression [24]. In our study, seven patients (35%) were negative for two or more proteins, and one patient (5%) was negative for all four proteins. One MSH2-negative patient was also negative for MSH6. Similarly, two MLH1-negative patients did not express PMS2. Further

genetic tests are highly recommended for these three patients.

Lynch syndrome is an autosomal dominant cancer. The lifetime risk of endometrial cancer for women with Lynch syndrome is approximately 40% to 60%, a level that equals or exceeds their risk of colorectal cancer. Prophylactic hysterectomy with bilateral salpingoophorectomy is an effective strategy for preventing endometrial and ovarian cancer in women with Lynch syndrome [25].

Atypical hyperplasia is known to increase the risk of endometrial cancer by about 30%. The cumulative 20-year progression risk among women who remain at risk for at least 1 year is less than 5% for non-atypical endometrial hyperplasia but is 28% for atypical hyperplasia [4].

The expression of MLH1, MSH2, MSH6, and PMS2 proteins is strongly associated with Lynch syndrome [26]. In the present study of 20 cases of complex endometrial hyperplasia, more than half of the patients showed loss of expression of at least one of the above proteins. The limitations of our study are 1) the small number in the study group, 2) not having a confirmative test, and 3) the inability to define the cut-off level of intensity and proportion for further genetic tests. The present study suggests the necessity for further large-scale screening for Lynch syndrome in patients with complex endometrial hyperplasia to facilitate the early diagnosis of malignancy.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

Table 2. Immunohistochemical analysis of the expression of MLH1, MSH2, MSH6, and PMS2 expression

	Intensity				Total (n=20)
	0	**1+**	**2+**	**3+**	
MLH1	2	6	9	3	
MSH2	1	5	3	11	
MSH6	7	3	5	5	
PMS2	10	5	5	0	

Nuclear staining was scored as either 3 (marked), 2 (strong), 1 (weak), or 0 (negative).
MLH1, mutL homolog 1; MSH2, mutS homolog 2; MSH6, mutS homolog 6; PMS2, postmeiotic segregation increased 2.

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