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Evaluation of DNA Mismatch Repair Protein Expression as a Preliminary Screening for Lynch Syndrome in Young Japanese Women with Endometrial Cancer

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Abstract

Background: Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer, is an autosomal dominant cancer syndrome. There is an urgent need to identify patients with Lynch syndrome among patients with endometrial cancer. We aimed to evaluate the efficacy of measuring DNA mismatch repair (MMR) expression in young Japanese endometrial cancer patients to differentiate sporadic and Lynch syndrome-associated tumors.

Methods: In a retrospective analysis of 50-year-old or younger endometrial cancer patients, 106 tumors were evaluated for MSH2, MSH6, PMS2, and MLH1 expression by immunohistochemistry. Samples lacking MLH1 were further examined by real-time PCR to evaluate hypermethylation of the MLH1 promoter. Clinical characteristics of patients with suspected Lynch syndrome were then evaluated.

Results: Among the 106 samples, 25 (23.6%) had reduced MMR protein expression; MLH1, MSH2 and MSH6 staining was negative in 14, 6 and 5 cases, respectively, while no samples were negative for PMS2. Among the 14 cases lacking MLH1 staining, 10 were found to be associated with MLH1 promoter hypermethylation. Therefore, 15 (14.2%) cases of suspected Lynch syndrome-associated endometrial cancer were found. These patients presented with a significantly lower body mass index and had more first-degree relatives diagnosed with a Lynch syndrome-associated cancer. Our cohort included three Lynch syndrome patients with known mutations, and tumor samples from these patients showed an absence of the specific MMR protein that was mutated.

Conclusions: Our results demonstrate that immunohistochemical analysis of tumor samples for MMR protein expression can identify patients with Lynch syndrome. With early detection, colorectal cancer outcomes might be drastically improved.

Keywords: Lynch syndrome; Endometrial cancer; Screening; Immunohistochemistry

Introduction

Lynch disorder (LS), an autosomal overwhelming condition otherwise called genetic nonpolyposis colorectal tumor, is an acquired issue connected to germline changes in the DNA bungle repair (MMR) qualities MLH1, MSH2, MSH6, and PMS2. The lifetime danger of ladies with LS to create colorectal tumor is 50 to 85%. Ladies with LS have a danger of 40 to 60% to create endometrial growth, and an expanded danger of up to 15% to create other related malignancies [1-4].

In about 5% of patients, genetic mutations cause endometrial cancer, which occurs 10 to 20 years before most sporadic cancers [5-7] for selecting individuals for LS testing; however, revised guidelines [8] recommend immunohistochemistry (IHC) or MSI testing for identification of patients with LS because the population of women with endometrial cancer and LS who fulfill Amsterdam II criteria can be as low as 30% [9].

Patients and their families meeting Amsterdam II criteria have 22% sensitivity and 98% specificity for diagnosis of LS [10]. Evidence of MSI or abnormal IHC in one of the MMR genes has been recommended as an indication for genetic testing [11].

The advantages of IHC for screening LS are that it is readily available for any pathology lab, inexpensive and, importantly, it directly identifies the affected gene [12,13]. In spite of the fact that screening for LS is for the most part being examined for colorectal disease in The United States and some European nations, it might likewise have esteem for patients with endometrial tumor; nonetheless, few reports concentrating on the adequacy of screening for LS in Japanese endometrial growth patients have been distributed [14].

In this study, as a preliminary step to applying these techniques to clinical practice, we retrospectively investigated IHC screening of LS from young endometrial cancer patients using surgical specimens.

Patients and Methods

This study was approved by the Institutional Review Board of Shikoku Cancer Center. The endometrial cancer cases were collected from the pathology files of Shikoku Cancer Center, and all patients met the following conditions: 50 years-old or younger, an initial treatment of hysterectomy without preoperative chemotherapy, and hysterectomy that was performed between 1 January 2000 and 31 December 2012. Clinical characteristics were reviewed from patient medical records; age, past history and family history were based on patient data at the time of surgery.

Our hospital has been undergoing genetic testing on the basis of family history at the division of familial cancer in clinical practice. Thus, several patients knew their germline mutational status with regard to LS from this genetic counseling; however, these genetic data were not transferred to us and did not play a role in this study. Under the study conditions, we only referred to our IHC results to investigate mutations in MMR genes.

IHC was done at the Department of Pathology, Shikoku Cancer Center. Staining of MMR protein expression was performed using HISTOSTAINER 36A (Nichirei Biosciences, Tokyo, Japan). Deparaffinized 4-μm-thick serial sections of a representative surgical specimen from each case were subjected to EDTA at 100°C for antigen retrieval. The sections were treated with primary antibodies against the mismatch repair proteins for 30 min. The antibodies used in this study were MSH6 (Clone EP49, 1:50 dilution, Dako, Kyoto, Japan), MSH2 (Clone FE11, 1:50 dilution, Dako), PMS2 (Clone EP51, 1:40 dilution, Dako) and MLH1 (Clone ES05, 1:50 dilution, Dako). The antibodies were visualized using Histofine Simple Stain MAX PO (Nichirei Biosciences, Tokyo, Japan) IHC results were evaluated by two gynecologic oncologists and one pathologist. An agreement of pathologic review was reached with one pathologist and at least one gynecologic oncologist. Judgment of IHC samples complied with the previous study directed by Grag et al. [15]. An estimation of MMR gene expression abnormalities was made according to Table 1.

MLH1	MSH2	MSH6	PMS2	Possible causes
−	+	+	−	Sporadic cancer
−	+	+	+	Germline mutation MLH1
+	+	+	−	Germline mutation of PMS2, rarely MLH1
+	−	−	+	Germline mutation of MSH2 or EPICAM, rarely of MSH6
+	−	+	+	Germline mutation of MSH2
+	+	−	+	Germline mutation of MSH6, less likely MSH2

Table 1: Possible causes of protein expression patterns from immunohistochemistry.

Hypermethylation of the CpG island within the MLH1 promoter correlates with an nonattendance of quality expression. To identify epigenetic adjustments of the MLH1 promoter locale, quantitative DNA methylation investigation utilizing constant PCR was performed in cases with no MLH1 expression. Bisulfite changed over DNA was set up from formalin-settled paraffin-inserted tissue tests utilizing EpiTect Fast Bisulfite Conversion Kit (QIAGEN, Hilden, Germany), and was enhanced utilizing the EpiTect Whole Bisulfite Conversion Kit and ABI GeneAmp PCR System 9700. Alluding to the study by Perez-Carbonell et al. [16], the collagen 2A1 (COL2A1) quality, which needs CpG islands in its amplicon succession was utilized to standardize the measure of info bisulfite DNA. Methylation-particular constant PCR was performed utilizing ABI7300 with TaqMan test and particular groundworks for MLH1 and COL2A1. The rate of methylated reference or level of methylation at a particular locus was ascertained with MLH1:COL2A1 proportion of a specimen separated by the MLH1:COL2A1 proportion of the complete methylated control DNA. We utilized a cut-off of 4% (>4% of methylated reference versus negative ones <4%), as has been accepted in past studies and broken down every example and dissemination of positive methylated specimens in triplicate. Clinical and pathologic valuables were compared between the two groups using Student's t-test and Chi-square tests. Statistical significance was considered as values of $P < 0.05$.

Results

Loss of MLH1, MSH2 and MSH6 protein expression was observed in 25 of 106 endometrial cancer cases from patients aged 50 years or younger. The loss of MSH6 protein expression in the cancer was observed in five cases; loss of MSH2 and MLH1 were observed in six and 14 cases, respectively. Loss of PMS2 protein expression was not observed (Table 2).

In cases where MLH1 protein expression was absent, MLH1 promoter hypermethylation was confirmed in 10 of 14 cases (Table 2).

The frequency of putative LS in Japanese endometrial cancer patients aged 50 years or younger was 14.2% (15/106) in our Study (Figure 1).

In the 15 cases of putative LS-associated endometrial cancer, the patient's BMI was significantly lower than the cases that were most likely sporadic endometrial cancer (Table 3).

In terms of family history of LS-associated cancer in at least one first-degree relative, putative LS endometrial cancer cases have more frequent incidents than the sporadic endometrial cancer cases.

Loss of MMR pattern	MLH1/PMS2	MLH1	PMS 2	MSH2/MSH6	MSH2	MSH6
No. cases	14	0	0	6	0	5
MLH1 hypermethylation	10	NA	NA	NA	NA	NA
Germline mutation identified	NA	NA	NA	2/2	NA	1/1
Abbreviation: NA: Not Available						

Table 2: Loss of MMR patterns among probable sporadic and putative LS endometrial cáncer.

Our study included three patients with known MMR gene mutations. Their IHC results showed a loss of MSH6 protein expression in one case and loss of MSH2 protein expression in the other two cases. Two cases with MHS2 mutation had loss of MSH2

protein expression, and the case with loss of MSH6 protein expression had a MSH6 variant of unknown significance (Table 2).

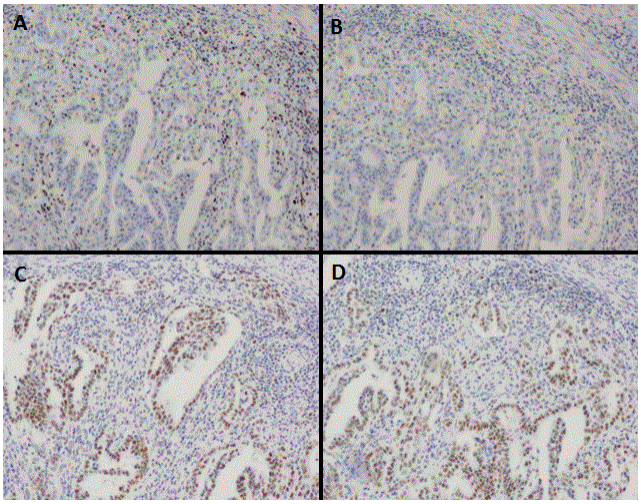

Figure 1: Lynch syndrome-associated endometrial carcinoma suspect of germline MSH2 mutation. Although the lymphocytes and stromal cells were stained positive, loss of nuclear MSH6 (A) and MSH2 (B) expression was observed. However, PMS2 (C) and MLH1 (D) showed diffuse nuclear expression. All images were taken at 10X magnification.

	IHC Results			
	Positive (n=15)	**Negative (n=91)**	**All Patients (n=106)**	*P*
Characteristic	**No. of Patients (%)**	**No. of Patients (%)**	**No. of Patients (%)**	
Age at diagnosis (years)				0.8203
Mean	44.4	44.5	44.4	
Median	47	47	47	
Range	32.0-50.0	30.0-50.0	30.0-50.0	
BMI at diagnosis				0.0021
Mean	20.9	25.3	24.7	
Median	21	24.6	23.4	
Range	17.6-25.5	17.7-39.7	17.6-39.7	
Patients with >1 Lynch syndrome-associated cancer				
Colon	1	1	2	
Ovarian	0	0	0	
Brain	0	0	0	
Lynch syndrome-associated cancer in at least one first-degree relative				0.0008
Parents				
Colon	4	8	12	
Endometrioid	2	1	3	
Ovarian	0	0	0	
Siblings				
Colon	2	0	2	
Endometrioid	0	1	1	
Ovarian	0	1	1	
Pathology				0.0519
Endometrioid adenocarcinoma	13	90	103	
Other	2	1	3	
Stage				0.4761*
I	7	67	74	
II	4	8	12	
III	4	12	16	
IV	0	4	4	
BMI: Body Mass Index, *P value between stage I/II and III/IV				

Table 3: Clinical and familial history of women aged 50 years or younger with endometrial cancer.

Discussion

This study was directed as a preparatory trial of clinical screening for LS among youthful endometrial growth patients, and uncovered two essential discoveries. In the first place, in Japanese endometrial growth patients matured 50 years or more youthful, MMR protein expression was missing in 23.6% of the cases and putative LS-related endometrial disease was found in 14.2% of the cases. Patients with suspected LS-related ailment had a lower BMI and positive family history contrasted and patients with suspected sporadic sickness. Second, screening strategies, for example, IHC for endometrial tumor examples are helpful and might be conceivable to interpret into clinical practice.

While tending to inherited hereditary transformations, moral contemplations are critical. On the off chance that screening for LS among patients with endometrial growth is to end up normal, we should first decide the recurrence of endometrial disease brought about by LS; notwithstanding, there are few reports [14] researching the recurrence of loss of MMR protein expression in endometrial tumor in Japan. As indicated by "Uterine Neoplasms" of the NCCN clinical practice rule in oncology (version 2, 2016), screening for hereditary transformations ought to be considered in all patients with endometrial malignancy however particularly in those more youthful than 50 years old [5,12,17,18]. Although the percentage of LS in

endometrial cancer patients of all ages is only 1.8% [19], for patients under 50 years old, the frequency increases up to 9% [20,21]. Low BMI, age less than 50 and positive family history have all been identified as risk factors in endometrial cancer patients who might benefit from LS screening [21-23]. In these circumstances, LS screening targeting young endometrial cancer patients is of great significance. In this study, we targeted endometrial cancer patients aged 50 years or younger to demonstrate the utility of LS screening from surgical specimens using IHC, and our data show that in Japanese endometrial cancer aged 50 years or younger, the frequency of absent MMR protein expression was 23.6% and putative LS-associated endometrial cancer was 14.2%. Our data also confirmed that putative LS-associated endometrial cancer patients had a significantly lower BMI and had a more positive family history. Sugawara et al. also described an efficient screening criteria that targeted an age of onset <50, or personal or family history of associated cancer for LS in Japan [14].

IHC screening from endometrial surgical specimen is useful and may be possible to do in clinical practice. LS screening using IHC for the MLH1/MSH2/MSH6/PMS2 proteins and/or testing for MSI as tests for MMR deficiency of newly diagnosed CRCs are recommended in recently published guidelines [10]. Similarly, these guidelines suggest that an individual who has a personal history of a tumor showing evidence of MMR deficiency (without evidence of MLH1 promoter methylation), or uterine cancer diagnosed before the age of 50 should undergo genetic evaluation for LS [10]. IHC has been shown to be an acceptable substitute for MSI as concordance rates between MSI and IHC are 94% in both CRC and endometrial cancer [24]. However, IHC provides additional information over MSI in that it allows gene specific DNA analysis depending on the staining pattern [25]. IHC does have limitations that have been pointed out in several situations; for instance, if the staining pattern suggests concurrent MLH1 and PMS2 deficiency it most likely indicates sporadic hypermethylation of MLH1 promoter (Table 2), although this pattern is also seen when there is a germline mutation in MLH1. Similarly, concurrent MSH2 and MSH6 deficiency usually indicates a germline mutation of MSH2 or EPICAM, rarely of MSH6.

In gynecological practices, IHC will be a more efficient means of LS screening than using the Amsterdam II criteria. Results of IHC can determine the loss of expression of MMR proteins in tumor tissue, and IHC testing reveals the absence of one or more MMR proteins, and estimates some possible causes (Table 2). Methylation of the MLH1 promoter, leading to a loss of MLH1 expression, is strongly associated with sporadic endometrial cancer. More than 70% of endometrial cancers that are MSI-high are due to MLH1 hypermethylation [26]. Somatic hypermethylation of MLH1 is an accurate and cost-effective pre-screening method in the selection of patients that are candidates for MLH1 germline analysis when LS is suspected and MLH1 protein expression is absent [27].

This study has two main limitations. The first is that the study included only a small number of patients at a single medical center. The second is the retrospective nature of the study, so we could not enforce genetic test on all cases suspected for LS, which may have introduced some bias. A multicenter study under uniform conditions may be able to provide further useful data on this subject.

In this study, we revealed that the frequency of absent MMR protein expression was 23.6% and suspected LS was 14.2% in Japanese endometrial cancer patients aged 50 years or younger, and IHC as a screening method for endometrial surgical specimen is useful and may be possible to translate into clinical practice.

Kwon et al. evaluated the expenses and advantages of various testing criteria to recognize LS in ladies with endometrial disease, finding that IHC triage of ladies with endometrial growth matured 50 years or more youthful is compelling second just to ladies with endometrial tumor at any age having no less than one first-degree relative with a LS-related malignancy. These two strategies seem to estimate the subsequent number of colorectal cancer cases equivalently. The study concluded that IHC triage of women with endometrial cancer at any age having at least one first-degree relative with a LS-associated cancer is a cost-effective strategy for detecting LS [12]. Our data suggest that in Japan we should triage women with endometrial cancer with IHC, and then offer them the opportunity to undergo genetic counseling and genetic testing. If we provide this information to patients and their families who are suspected to have LS, genetic tests and surveillance of high-risk patients will be provided. Together, our data show the usefulness of a diagnostic strategy for LS based on routine analysis for MMR protein expression in endometrial cancer.

Disclosure Statement

The authors declare that they have no conflict of interest.

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