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Lynch Syndrome in Women Less Than 50 Years of Age With Endometrial Cancer

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Abstract

OBJECTIVE—To estimate the frequency of mismatch repair deficiencies associated with hereditary nonpolyposis colorectal cancer, or Lynch syndrome, in women less than age 50 with endometrial cancer.

METHODS—Consecutive patients less than age 50 diagnosed with endometrial adenocarcinoma were identified. Available pathologic specimens were freshly sliced, and protein expression for MLH1, MSH2, MSH6, and PMS2 was evaluated by immunohistochemistry. Slides were scored on a semiquantitative method with complete absence of any of the four proteins suggesting a deficiency. All results were confirmed by microsatellite instability testing.

RESULTS—Sixty-one pathology specimens were analyzed. Twenty-one (34%) of the tumors had absence of staining of at least one of the four mismatch repair proteins determined by immunohistochemistry and confirmed by microsatellite instability testing. Obese patients were less likely than nonobese patients to have a mismatch repair deficiency (21% versus 59%, respectively). Non-obese patients had a relative risk for a mismatch repair deficiency of 5.5 (95% confidence interval 1.6–19.1; $P=.01$).

CONCLUSION—Many women diagnosed with endometrial cancer before age 50 will have a mismatch repair deficiency discovered by immunohistochemistry and microsatellite instability testing. A number of young women diagnosed with endometrial cancer will require further genetic testing for mismatch repair mutations.

Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome is the most common hereditary cancer syndrome in the United States and Europe. More than 50% of women diagnosed with Lynch syndrome will present with a gynecologic malignancy as their sentinel cancer; the majority of these cancers will be endometrial cancer.¹ HNPCC is caused by a germline mutation in the DNA mismatch repair mechanism that works to ensure the fidelity of the genome during cell division.² The most common mutations are MLH1, MSH2, MSH6, and PMS2; defects in these genes result in microsatellite instability.³ Per the Modified Bethesda criteria recommendations, patients less than age 50 diagnosed with colorectal cancer should undergo testing to determine whether they have a genetic mutation associated with Lynch syndrome (see the Box). Although approximately 11% of patients

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under the age of 50 diagnosed with endometrial cancer will have a mutation consistent with Lynch syndrome,⁴ no recommendations for genetic testing exist specific to endometrial cancer.⁵

The purpose of this study was to estimate the frequency of mismatch repair deficiencies detected by immunohistochemistry and microsatellite instability testing in women less than age 50 with endometrial cancer. Additionally, we sought to evaluate whether obesity affected the frequency of mismatch repair deficiencies.

METHODS

After institutional review board approval from the University of Alabama at Birmingham, consecutive patients less than age 50 diagnosed with endometrial adenocarcinoma between 1996 and 2006 were identified using a computerized database. All patients had undergone total hysterectomy, bilateral salpingo-oophorectomy, and surgical staging for endometrial adenocarcinoma at our institution. Only patients less than age 50 with available p35 paraffin-embedded tissue samples were eligible for this study. Fifty-seven percent of the patients had nodal tissue available. Fresh hematoxylin and eosin-stained slides were sliced, and a diagnosis of primary endometrial adenocarcinoma was confirmed by a gynecologic pathologist.

Tissue sections (5 micrometers) of both malignant tissue and representative normal tissue were sliced and mounted on Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA) for staining and interpretation (Fig. 1). Sections were cut 1 to 2 days before immunostaining to avoid potential problems in antigen recognition due to storage degradation of cut tissue sections on glass slides.^{6,7} Immunostaining was performed as described in our earlier studies of antigen expression in different tissues.^{8,9} In brief, sections were melted and incubated overnight at 37°C before staining. The tissue sections were deparaffinized in xylene and subsequently rehydrated in graded alcohols. Heat-induced antigen retrieval was performed on the sections for 20 minutes. Each section was incubated with 3% goat serum at room temperature for 1 hour to reduce non-specific immunostaining. Tissue sections then were incubated with anti-human mouse MLH1 (clone G168-728; dilution 1:50; BD Pharmingen, San Diego, CA), MSH2 (clone FE11; dilution 1:100; Calbiochem, La Jolla, CA), MSH6 (clone 44; prediluted; Zymed, San Francisco, CA) and PMS2 (clone A16-4; dilution 1:50; BD Pharmingen, San Diego, CA) primary monoclonal antibodies. Sections on which the primary antibody was not applied were utilized as negative controls.

Slides were analyzed and scored with a semiquantitative method (0 to +3 scale) based on degree of intensity and percentage of cells stained. Complete absence of staining for MLH1, MSH2, MSH6, or PMS2 indicated a mismatch repair deficiency. The slides were analyzed independently by two pathologists who were blinded to patients' demographics and clinical information. In addition to normal tissue slices, adjacent normal stroma and lymphocytes within cancer specimens served as internal controls for each case for proper staining.

A second series of tissue sections were obtained from the same paraffin-embedded blocks of all specimens and were evaluated for microsatellite instability by using a multi-species detection system (Signet Laboratory Inc., Dedham, MA). The sections were exposed to the previously mentioned antibodies, followed by biotinylated multispecies antibodies including anti-mouse antibodies for 20 minutes, and then incubated with peroxidase-labeled streptavidin for 20 minutes. A diaminobenzidine tetrachloride super-sensitive substrate kit (Bio-Genex, San Ramon, CA) was used to visualize the antibody-antigen complex. Sections were counterstained with hematoxylin. Tumors were described as microsatellite instability-

high when two or more markers were identified and as microsatellite instability- low when zero to one marker showed microsatellite instability.

Patient demographics and clinicopathologic factors were obtained and compared with degree of immunostaining. Categorical data were compared using χ^2 test or Fisher exact test, and continuous variables were compared using Student's *t* test. All statistical calculations were performed using SPSS 11 Software (Chicago, IL).

RESULTS

Sixty-one patients had available paraffin block tissue that was sufficient to perform immunohistochemical staining and microsatellite instability testing (Table 1). All patients were less than age 50 at the time of diagnosis and had primary endometrial adenocarcinoma of endometrioid histologic subtype. The mean age was 43 (range 33–49), and the mean BMI was 38.1 (range 18–76). The majority of the patients were obese (BMI more than 30), Caucasian, and had a stage I cancer. Nodal tissue was available for 35 (57%) of the patients.

Twenty-one patients (34%) had immunohistochemistry and microsatellite instability findings consistent with a mismatch repair deficiency. The most common were MSH6 (n=16), PMS2 (n=5), and MLH1 (n=3) (Table 2). The majority of patients (77%) had only one deficiency, with four patients having two deficiencies. Patients with absence of staining were similar to patients with normal staining in regard to age, stage, and grade of tumor (Table 3). Patients with mismatch repair deficiencies were more likely to be Caucasian. (*P*=.03).

In our cohort, obese patients were less likely to have a mismatch repair deficiency (21%) compared with non-obese patients (59%) (*P*=.005). Mean BMI was 32 for patients with mismatch repair deficiencies, compared with 41 for patients without a mismatch repair deficiency (*P*=.02). This finding was further confirmed when patients were stratified by weight groups (*P*=.04). This relationship led to an RR of non-obese patients under the age of 50 with endometrial cancer having a mismatch repair deficiency of 5.5 (95% confidence interval 1.6–19.1; *P*=.01).

DISCUSSION

The Modified Bethesda criteria recommend that patients less than 50 years of age diagnosed with colorectal cancer undergo immunohistochemistry or microsatellite instability screening followed by genetic testing to detect whether a germline mutation-associated Lynch syndrome exists. Although Lynch syndrome traditionally has been associated with early-onset colorectal cancer, more than one half of women diagnosed with Lynch syndrome will present with a gynecologic malignancy as their sentinel cancer.¹ Additionally, the average age for the development of endometrial cancer in patients with Lynch syndrome is 48, approximately 10 years younger than in the general population.¹⁰ Although estimates of the rate of Lynch syndrome in young endometrial cancer patients (under age 50) are approximately 11%, no recommendations for screening exist specifically for this patient population.⁴

With the diagnosis of Lynch syndrome, the lifetime risk of developing a malignancy is as high as 70% in women, with numerous patients developing a secondary malignancy.¹⁰ A number of authors have suggested that patients with Lynch syndrome may benefit from increased cancer screening and surveillance to prevent secondary malignancies and thus improve survival.^{11–13} Recent guidelines have recommended early colonoscopic surveillance, endometrial sampling, transvaginal ultrasound, and urinalysis for patients with Lynch syndrome in an effort to detect secondary malignancies as soon as possible.

Additionally, prophylactic subtotal colectomy and hysterectomy with salpingo-oophorectomy also have been recommended in certain situations.¹⁴ Considering that this intense screening methodology improves detection and potentially survival, properly identifying patients at risk for Lynch syndrome is of utmost importance.

The current recommended screening for Lynch syndrome involves a stepwise progression of microsatellite instability testing followed by immunohistochemistry. Patients with a mismatch repair deficiency detected with immunohistochemistry should be referred for genetic testing to confirm a germline mutation. Because many patients with an MSH6 mutation do not exhibit microsatellite instability, we chose to utilize a more sensitive method to diagnose mismatch repair defects (performing immunohistochemistry first followed by microsatellite instability testing confirmation).^{15,16} Of note, it has been shown that a number of patients with PMS2 and MLH1 deficiencies will not have a germline mutation consistent with Lynch syndrome.^{17,18} In addition, MLH1 may be affected in sporadic cases secondary to hypermethylation, which is not caused by a germline mutation.

In our study, 34% of patients were found to have immunohistochemical evidence of a mismatch repair deficiency that would warrant genetic testing. Although obese patients (BMI more than 30) are considered to be at the highest risk for sporadic endometrial cancer related to their endogenous estrogen levels, a significant number of obese patients (21%) in our study had a mismatch repair deficiency. Importantly, the group at the highest risk was non-obese patients, with 59% of patients having a mismatch repair deficiency via immunohistochemistry and microsatellite instability testing. Irrespective of the definition of obesity (BMI more than 30, mean BMI, or obesity stratification), non-obese patients were more likely to have a mismatch repair deficiency.

The major weakness of this retrospective study is that we were unable to perform genetic testing to confirm a germline mutation in the patients with a mismatch repair deficiency. Although the sensitivity of immunohistochemistry to detect mismatch repair deficiency is high, specificity is much lower. Immunohistochemistry protein staining may be absent for various reasons (eg, genetic, epigenetic). Therefore, a prospective trial including formal genetic testing should be conducted in a cohort of young endometrial cancer patients to confirm these findings. Despite these limitations, these data suggest that a large number of young women will require formal genetic testing due to mismatch repair deficiencies detected by immunohistochemistry and microsatellite instability testing.

Based on the findings in our study, an increased awareness of Lynch syndrome in young endometrial cancer patients should be emphasized to health care providers. It is imperative that physicians obtain a thorough family history and provide referral for genetic counseling to those patients at risk for Lynch syndrome. Furthermore, patients diagnosed with Lynch syndrome should have regular cancer screening, including early colonoscopic surveillance, endometrial sampling, transvaginal ultrasound, and urinalysis. We recommend that all women less than age 50 diagnosed with endometrial cancer should have immunohistochemistry followed by microsatellite instability testing. Women who have mismatch repair defects detected by these methods should undergo germline mutation testing. If additional research confirms a high rate of mismatch repair mutations in young women with endometrial cancer, the Modified Bethesda criteria should implicitly state that patients with colorectal or endometrial cancer diagnosed before age 50 should be tested for Lynch syndrome.

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Modified Bethesda Criteria

Tumors from individuals should be tested for microsatellite instability in the following situations:

1. Colorectal cancer diagnosed in a patient who is less than 50 years of age.
2. Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumors,* regardless of patient age.
3. Colorectal cancer with the microsatellite instability-H-like histology diagnosed in a patient who is less than 60 years of age.
4. Colorectal cancer diagnosed in a patient with one or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed before the age of 50.
5. Colorectal cancer diagnosed in a patient with two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age.

*HNPCC-related tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain tumors; sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome; and carcinoma of the small bowel.

Fig. 1.
Representative immunohistochemical staining of mismatch repair mutations.

Table 1**Patient Demographics**

Age, y	
Mean	43
Range	33–49
BMI, kg/m ²	
Mean	38.1
Range	18–76
Obese (BMI greater than 30)	
Yes	39 (61)
No	22 (39)
Obesity strata	
Underweight (18.4 or less)	1 (2)
Normal weight (18.5–24.9)	8 (15)
Overweight (25.0–29.9)	8 (15)
Obese (30.0–34.9)	10 (17)
Morbid obesity (35.0 or more)	29 (51)
Race	
Caucasian	48 (80)
African American	13 (20)
Stage	
Ia	6 (10)
Ib	44 (74)
Ic	2 (3)
IIa	1 (2)
IIb	2 (3)
III	4 (6)
IV	1 (2)
Grade	
1	21 (35)
2	34 (58)
3	4 (7)

BMI, body mass index.

Data are presented as n (%) unless otherwise noted.

Table 2

Frequency of Mismatch Repair Mutations

PT	MLH1	MSH2	MSH6	PMS2
1	+	-	-	-
2	-	-	+	-
3	-	-	+	-
4	-	-	+	-
5	-	-	+	-
6	-	-	+	-
7	-	-	+	-
8	-	+	+	-
9	-	-	+	-
10	-	-	+	-
11	+	-	-	-
12	-	-	+	-
13	-	-	-	+
14	-	-	+	-
15	+	-	-	+
16	-	-	+	+
17	-	-	-	+
18	-	-	+	-
19	-	-	+	-
20	-	-	+	+
21	-	-	+	-

PT, patient; +, mismatch repair defect detected; -, no mismatch repair defect detected.

Table 3

Impact of Mismatch Repair Mutation Status on Clinicopathologic Factors

Characteristic	Positive MMR Mutation (95% CI)	Negative MMR Mutation (95% CI)	P
Age (mean)	43.1 (40.5–45.3)	43.2 (41.7–45.1)	.92
BMI	32.0 (25.8–38.2)	41.0 (36.8–45.5)	.02
Obese (BMI greater than 30)			.005
Yes	8	31	
No	10	7	
Obesity strata			.04
Underweight (18.4 or under)	0	1	
Normal weight (18.5–24.9)	5	3	
Overweight (25.0–29.9)	5	3	
Obese (30.0–34.9)	3	7	
Morbid obesity (35.0 or higher)	5	24	
Race			.03
Caucasian	20	29	
African American	1	11	
Stage			.24
Ia	5	2	
Ib	15	30	
Ic	–	2	
IIa	1	–	
IIb	–	1	
III	–	4	
IV	–	1	
Grade			.41
1	9	12	
2	9	23	
3	3	3	

MMR, mismatch repair; CI, confidence interval; BMI, body mass index.