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Women with double primary cancers of the colorectum and endometrium: do they have Lynch syndrome?

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ABSTRACT

Objective: The aim of this study was to determine the clinical characteristics of women with double primary cancers of the colorectum and endometrium and assess the probability of Lynch syndrome.
Study design: We identified 15 women with paraffin-embedded blocks available who were diagnosed, treated and followed for double primary colorectal and endometrial cancers at in a single institution between 1994 and 2014. If there was a family history that met the revised Amsterdam criteria for Lynch syndrome, the woman was considered to have 'clinically defined Lynch syndrome'. If immunohistochemical (IHC) loss of expression of mismatch repair genes (*MLH1, MSH2, MSH6, or PMS2*) or high microsatellite instability (MSI) was demonstrated in molecular testing, the case was considered 'suspected Lynch syndrome'.
Results: The incidence of clinically defined Lynch syndrome according to the revised Amsterdam criteria was 66% (8 of 15). All 8 of the women clinically diagnosed with Lynch syndrome had either abnormal IHC loss or MSI-high, indicating a suspected Lynch syndrome. Furthermore, 27% (4 of 15) experienced second primary colorectal cancer or other Lynch syndrome-related cancers. Overall, 66% (10 of 15) met the criteria for clinically defined Lynch syndrome or suspected Lynch syndrome.
Conclusions: Based on our findings, a large percentage (66%) of women with double primary cancers of the colorectum and endometrium are likely to be diagnosed with Lynch syndrome.

Introduction

Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC), is the most common form of autosomal-dominant inherited cancer susceptibility syndrome, accounting for up to 6% of all colorectal and endometrial cancer cases [1,2]. This hereditary syndrome usually results from a germline mutation in 1 of 4 DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, *or PMS2*). Women who carry a mutation in these genes have a 39–54% lifetime risk of colorectal cancer and a 40–60% lifetime risk of endometrial cancer [3–5]. Up to 25% of individuals with Lynch syndrome have a second, metachronous cancer within 10 years after the diagnosis of their first cancer, and 50% by 15 years after the first cancer [6,7]. Such double primary cancers of the colorectum and endometrium are a tragedy for the patient and an economic burden for the family and country. Therefore, a significant amount of information about double primary cancers and Lynch syndrome is needed in order to identify such individuals and families in managing future cancer risks.

The aim of this study was to determine the clinical characteristics of women with double primary cancers of the colorectum and endometrium who were diagnosed and treated at a single institution during the last 20 years and to evaluate the immunochemical (IHC) staining and microsatellite instability (MSI) for Lynch syndrome in such women.

Materials and methods

After obtaining approval from the institutional review board, we included patients who were diagnosed, treated and followed

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for double primary cancers of the colorectum and endometrium at Samsung Medical Center between 1994 and 2014 from the Tumor Registry database for this study. Exclusion criteria were (1) metastatic colorectal or endometrial cancer from another site and (2) paraffin-embedded tumor blocks of the colorectum and endometrium were not available for molecular analysis. Family history was evaluated using the medical records to determine site of cancer, age of diagnosis and relationship with patient. However, when medical records were incomplete, telephone interviews were done. Based on family history, patients were classified into two groups depending on whether or not they had clinically defined Lynch syndrome according to the revised Amsterdam criteria [8] for clinical diagnosis of Lynch syndrome. Double primary cancers were classified into two types according to the timing of cancer detection: synchronous tumor and metachronous tumor. If a second tumor was discovered within 6 months of resection of the first, it was classified as a synchronous tumor not detected at operation. A second tumor developing more than 6 months after the first was classified as a metachronous tumor.

IHC staining was performed on both colorectal and endometrial tumor specimens to determine the protein expression of 4 MMR genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*). 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). Mouse monoclonal antibodies against MLH1 (Novocastra Laboratories, Newcastle, UK), MSH2 (Novocastra Laboratories), MSH6 (BD Transduction Pharmingen, San Jose, CA, USA), and PMS2 (Zytomed Systems, Berlin, Germany) were used. Immunostaining was performed with Bond-Max (Leica Biosystems, Newcastle, UK) according to the manufacturer's instructions. The normal staining patterns for MLH1, MSH2, MSH6, and PMS2 were nuclear. Loss of expression of MLH1, MSH2, MSH6, and PMS2 in cancer cells was demonstrated by the total absence of nuclear staining in the tumor. Adjacent normal stoma or infiltrating lymphocytes served as an internal positive control for each case. Nuclear staining was scored as either positive (indicating intact expression) or negative (indicating loss of expression) compared with the corresponding internal control.

MSI testing was performed only in the colorectal specimen. Tumor cells were microdissected by hand from adjacent normal tissue to insure that each tumor sample contained at least 70% neoplastic cells. Normal tissue from the same patients was also analyzed as a control. DNA was extracted from both tumor and normal tissue for each sample. After DNA amplification using fluorescent-labeled primers, a panel of five microsatellite markers recommended by the National Cancer Institute (BAT25, BAT26, D5S346, D17S250, and D2S123) was analyzed for allelic shift using a multiplex fluorescence-based polymerase chain reaction (PCR) assay [9]. Amplified PCR products were then analyzed on an ABI PRISM 310 Genetic Analyzer (PerkinElmer Applied Biosystems, Foster City, CA, USA), using the GeneScan Analysis software provided by the manufacturer. Tumors showing allelic shift at two or more markers were considered MSI-high, whereas tumors showing allelic shift at only one marker were classified as MSI-low. Tumors with no allelic shift were classified as microsatellite-stable (MSS).

Once all data were collected, the likelihood of each patient having Lynch syndrome was determined based on clinical and molecular criteria. If the patient had a family history that met the revised Amsterdam criteria [8], the patient was considered to have 'clinically defined Lynch syndrome'. If loss of MMR protein expression or high MSI was demonstrated in molecular testing, the case was considered 'suspected Lynch syndrome'.

Results

During the study period, we identified 15 patients with paraffin-embedded blocks available who were diagnosed, treated and followed for double primary cancers of the colorectum and endometrium at our institution. The demographic characteristics of for these 15 patients are listed in Table 1. The median age and body mass index of the patients at the diagnosis of double primary cancers of the colorectum and endometrium were 54 years (range, 47–69 years) and 23.4 kg/m^2 (range, 19.8–27.2 kg/m^2), respectively. The study patients were classified into two groups according to the timing of cancer detection: two (13%) had synchronous cancers and 13 (87%) had metachronous cancers. Of the 13 women with metachronous cancers, 6 (46%) had endometrial cancer diagnosed first and 7 (54%) were initially diagnosed with colorectal cancer. In cases of women with metachronous cancers, the median age at diagnosis of the first cancer and the median time to the second cancer were 53 years (range, 41–68 years) and 35.2 months (range, 7.1–113.0 months), respectively. Based on family history, 9 patients (60%) were considered to have clinically defined Lynch syndrome and the other 6 (40%) did not have clinically defined Lynch syndrome according to the revised Amsterdam criteria for the clinical definition of Lynch syndrome. There were 3 patients (20%) with additional Lynch syndrome-related cancer(s) outside of the colorectum and endometrium: two patients (case #3 and #14 in Table 3) had stomach cancer and one patient (case #15) had urothelial cancer and brain glioblastoma. Two patients (case #9 and #15) had a second primary colorectal cancer and the time intervals from the diagnosis of a first primary colorectal cancer were 47.2 months and 96.3 months, respectively. During the median follow-up period of 67.9 months (range, 20.7–157.8 months), one patient (case #5; 7%) experienced a recurrence of colorectal cancer in the lung parenchyma, underwent wedge resection by video-assisted thoracic surgery (VATS) followed by chemotherapy, and was alive without disease at the time of analysis.

Table 2 shows the pathologic characteristics of the endometrial and colorectal cancer. The most common histology of endometrial cancer was endometrioid (73%). Other histologies of endometrial

Table 1
Demographic characteristics ($n = 15$).

Characteristics	*N* (%) or median (range)
Ethnicity	
Asian	15 (100%)
Other	0
Age (year)[a]	53 (41–68)
Body mass index (kg/m^2)	23.4 (19.8–27.2)
Timing of cancer detection	
Synchronous cancer	2 (13%)
Metachronous cancer	13 (87%)
Colorectum first	7
Endometrial first	6
Time to second cancer (months)[b]	35.2 (7.1–113.0)
Clinical definition of Lynch syndrome[c]	
Met	9 (60%)
Not met	6 (40%)
Three or more Lynch syndrome-related cancers[d]	3 (20%)
Secondary primary colorectal cancer	2 (13%)
Follow-up period (months)	67.9 (20.7–157.8)
Disease recurrence	1 (7%)

[a] At the diagnosis of first cancer.
[b] In cases of metachronous cancers.
[c] Indicates individuals who met revised Amsterdam criteria for clinical definition of Lynch syndrome.
[d] Lynch syndrome-related cancers include colorectal, endometrial, gastric, ovarian, pancreas, ureter and renal pelvis, biliary tract, brain glioblastoma, and small intestinal cancers, as well as sebaceous gland adenomas and keratoacanthomas as seen in Muir-Torre syndrome.