Received: November 11, 2023
Accepted: December 25, 2023

Acad Med J 2023;3(3):67-72
UDC: 616-056.7:575.224.4]:618.14-006.6
DOI: https://www.doi.org/10.53582/AMJ2333067ks
Case report

DETECTION OF LYNCH SYNDROME IN ENDOMETRIAL CANCER PATIENTS

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Abstract

Lynch syndrome (LS) is an autosomal dominant inherited disease defined by germline mutations in mismatch repair (MMR) genes, leading to a defective DNA MMR system. Patients with LS have predisposition to a spectrum of cancers, primarily colorectal cancer, but LS-associated endometrial cancer (LS-EC) is the most common extraintestinal cancer and occurs in 2% of LS patients. The most frequently mutated MMR genes are MLH1, MSH2, MSH6 and PMS2. Clinico-pathologic features of LS-EC are: early age of onset, lower body mass index, endometrioid type of carcinoma and lower uterine segment involvement.

Recent studies support LS screening in every EC patient since MMR status is also part of the molecular subclassification of endometrial cancers. Screening methods include traditional clinical criteria and molecular techniques, such as MMR-immunohistochemistry (MMR-IHC), microsatellite instability (MSI) testing, MLH1promoter methylation testing and gene sequencing. MSI can also be detected in sporadic tumors, through epigenetic events inactivating the MMR system.

Patients with diagnosed LS and their affected relatives should be closely monitored in order to prevent the development of other types of cancer. Patients with advanced recurrent microsatellite instability-high (MSI-H)/mismatch repair-deficient (dMMR) endometrial cancer can also benefit from immunotherapy.

We describe our 3-year experience in screening of Lynch syndrome in EC patients.

Keywords: Lynch syndrome, endometrial cancer

Introduction

Lynch syndrome is one of the most common hereditary cancer syndromes[1]. It confers a markedly increased lifetime risk of colorectal cancer, endometrial cancer, as well as cancers of the ovary, stomach, urothelial tract, small bowel, pancreas, biliary tract, and sebaceous neoplasms of the skin[1]. Women with Lynch syndrome have an

increased risk of developing endometrial cancer (up to 60%), which is the sentinel diagnosis in approximately one-half of the cases[2]. The molecular phenotype of microsatellite instability was discovered in 1993 and was subsequently linked with Lynch syndrome-associated colorectal cancer[3].

Lynch syndrome is a consequence of mutation in any of the mismatch repair genes, involved in DNA mismatch repair. This mutation most often leads to a loss of protein expression, and thus to genome instability in the cells. The primary diagnostic strategy for LS is germline MMR gene analysis in individuals with tumors demonstrating high-level MSI (MSI-H) and/or deficient MMR protein expression[3].

The cumulative risk of cancer for LS patients at the age of 70 years is over 40%[4]. In 50% of the LS cases, endometrial cancer is often the first cancer that occurs and reveals the familial predisposition to cancer[4].

In contrast to sporadic endometrial cancer, LS-EC usually occurs before the age of 60 and in women with low body mass index[3]. Patients with LS-associated colorectal cancer have better prognosis[5], but the association of LS and prognosis in endometrial cancer is not well established[6]. It seems that tumors in younger patients behave more aggressively[4]. Additionally, LS-EC can also be synchronous with ovarian cancer.

Material and methods

This retrospective study includes cases of endometrial cancer surgically treated in our hospital in the period from January 2020 till November 2023. All cases, regardless of the age at presentation, were screened for Lynch syndrome.

For this purpose, we used the MMR panel of ready-to-use antibodies from Ventana (Ventana Medical Systems, Oro Valley Arizona, United States), comprising 4 antibodies against protein products of the four mismatch repair genes: MLH1 (clone M1), PMS2 (cloneA16-4), MSH2 (clone G219-1129) and MSH6 (clone SP93). All cases were first analyzed with PMS2 and MSH6 antibodies. Then, if there was a loss of expression of the PMS2 antibody, additional slides were analyzed with MLH1 antibody. On the other hand, if MSH6 staining was missing, additional slides were analyzed with MSH2

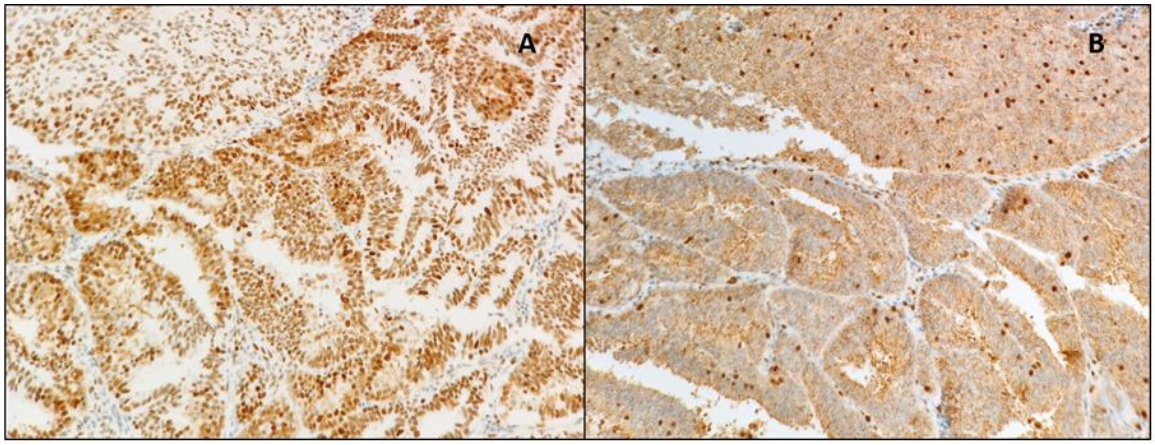

Fig. 1. Presence of unequivocal nuclear staining for MMR antibody is considered as intact expression (A), whereas loss of nuclear staining in tumor cells with intact nuclear staining in the surrounding stromal or immune cells (B) is considered as loss of expression

antibody. All cases with loss of expression of antibodies MLH1 and PMS2 were send to a referral molecular laboratory for MLH1 gene promoter methylation analysis. Cases with loss of expression of any of the antibodies and negative result from the gene promoter methylation analysis were sent for genetic counseling and additional genetic testing for Lynch syndrome.

After standard tissue processing and paraffin embedding, 4 micron-thin sections were cut. Target retrieval, primary antibody and detection protocols were applied as recommended by the vendor. Slides were counterstained with hematoxylin and eosin, and microscopic analysis was performed by an experienced pathologist. Cases were considered to have intact protein expression when tumor cells exhibited unequivocal nuclear positivity (Figure 1).

Results

Seventy-eight patients were surgically treated for endometrial cancer in the selected period. The youngest patient was 16 years old, whereas the oldest patient was 82 years old (mean age of patients at presentation was 62.7). Six of the cases were less than 50 years old, comprising 7.7% of the cases.

Table 1. Gene and age distribution of MMR deficient endometrial cancer cases

		MMR genes protein expression			
Case No.	**Age at presentation**	**MLH1**	**PMS2**	**MSH2**	**MSH6**
1	53	-	-	+	+
2	68	-	-	+	+
3	78	-	-	+	+
4	64	-	-	+	+
5	69	-	-	+	+
6	61	-	-	+	+
7	70	-	-	+	+
8	73	-	-	+	+
9	72	-	-	+	+
10	68	-	-	+	+
11	78	-	-	+	+
12	76	-	-	+	+
13	50	-	-	+	+
14	53	-	-	+	+
15	46	+	+	+	-
16	47	+	+	+	-
17	44	+	+	+	-
18	67	-	-	-*	-*

*only in some cell clones

Results of the immunohistochemistry analysis showed intact protein expression of the analyzed MMR genes in 60 cases (76.9%). Loss of expression of both MLH1 and PMS2 genes was found in 14 cases (18%). In all cases the subsequent molecular analysis revealed presence of MLH1 gene promoter methylation. Furthermore, loss of expression of MSH6 gene was found in 3 cases (3.8%), and these patients were sent for additional genetic testing for Lynch syndrome.

All cases with sporadic MMR deficient endometrial cancer were 50 years or older (mean age 71.4). Conversely, cases with loss of expression of MSH6 gene were 47, 46 and 44 years old (mean 45.6).

In one case (1.3%) of dedifferentiated endometrial carcinoma, all tumor cells showed loss of expression of MLH1 and PMS2 genes, whereas in the poorly differentiated clone of cells loss of expression of MSH2 and MSH6 was also found (Table 1). This case was also sent for additional genetic testing for Lynch syndrome.

There was no difference in the morphology or localization of the neoplasms between the MMR proficient and MMR deficient endometrial cancer groups.

Discussion

Lynch syndrome was discovered almost 100 years ago by the father of cancer genetics, the American pathologist Aldred Scott Warthin, and was further described by Henry Lynch, the father of hereditary cancer[7]. The syndrome was first named hereditary nonpolyposis colorectal cancer as colorectal cancer seemed the most prevalent, but now it is known that uterine cancer and several other malignancies are also part of the Lynch syndrome spectrum[8,9].

Lynch syndrome has an incidence of 3% in colorectal cancer patients and 1.8% in EC patients[10]. In our series, possible Lynch syndrome was detected in 5.1% of cases. However, these 4 patients have to be further analyzed in order to confirm presence of Lynch syndrome, since other epigenetic events could possibly lead to gene silencing. However, other authors report that inherited cancer syndromes, Lynch syndrome being the most common one, are responsible for the occurrence of 5% of endometrial cancers[11-13]. The most prevalently affected gene product in our study was MSH6. According to the literature, MSH6 mutations are prevalent in endometrial cancers, occur at older age and are associated with markedly lower cancer risks than MLH1 or MSH2 mutations[7,14].

There are also patients who have pathogenic variants in more than one MMR gene, the so-called digenic LS. It is not clear whether digenic LS is more severe than LS due to a pathogenic variant in one gene, but has clear implications for clinical genetic counselling[15]. In our group of patients, we found one case of complete loss of expression of all four MMR genes in a distinct clone of dedifferentiated cells. This case was submitted to further molecular analysis in order to determine the cause of this event.

Current guidelines for Lynch syndrome detection in endometrial cancer patients rely either on risk evaluation, based on personal/family history, or detection of MMR deficiency on tumor tissue[16]. Immunohistochemistry is a preferred method for Lynch syndrome screening in many laboratories due to its efficiency, relatively lower price and technical demands in comparison to other molecular techniques[17]. Absence of MMR protein expression in tumor cells with retained expression in adjacent stromal cells indicates a defect in DNA mismatch repair. When MLH1 gene is mutated or silenced, cells exhibit loss of expression of both MLH1 and PMS2 genes, while patients with mutated or silenced MSH2 gene exhibit loss of protein expression of MSH2 and MSH6 genes. This phenomenon is due to the dominant role of MLH1 and MSH2 in heterodimer formation during mismatch repair[18]. PCR-based techniques also play an important role, moreover because MLH1 methylation analysis can identify women who likely have sporadic

endometrial cancer. However, some authors have detected high levels of microsatellite instability in tumors with retained expression of mismatch repair proteins[18].

Our results showed that 100% of the cases with loss of MLH1 and PMS2 protein expression had an epigenetic event, such as gene promoter methylation. Similar results are presented in the literature, where some authors report that up to 97% of the cases with loss of MLH1 gene protein expression have methylation of the MLH1 promoter region[18].

The mean age of patients with possible LS in our study was 45.6 years, similar to other studies in which the mean age of patients with LS was 49[11,19].

Currently, there are no data to suggest that the prognosis for women with Lynch syndrome-associated endometrial cancers is either better or worse than for women with sporadic cancers[6].

Conclusion

Using immunohistochemistry as an efficient and readily available screening method, we detected 4 possible cases of Lynch syndrome in endometrial cancer patients. Since endometrial cancer is often the sentinel cancer in these patients, who are at increased risk of developing cancers on other organs, carefully planned surveillance strategies and genetic counseling are of utmost importance.

Conflict of interest statement. None declared.

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