Two-Antibody Staining Method, A Cost-Saving Strategy for Universal Lynch Syndrome Screening in Endometrial Cancers

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ABSTRACT

Objective: Lynch syndrome is an autosomal dominant disorder that increases the risk of cancers in many sites. In women, endometrial cancer is often a sentinel tumor and thus immunohistochemistry for mismatch repair (MMR) proteins MLH1, MSH2, MSH6 and PMS2 is encouraged as a screening test. To reduce cost, staining for only 2 MMR proteins PMS2 and MSH6 has been proposed. This study aimed to determine whether a 2-antibody staining test is enough to screen for Lynch syndrome in endometrial cancer patients.

Materials and Methods: Cases of endometrial carcinoma with immunohistochemistry for 4 MMR proteins were reviewed. Results of immunohistochemistry screening were compared between all four antibodies and only two (PMS2 and MSH6) antibodies.

Results: Loss of expression of any MMR proteins was detected in 51 out of 203 cases (25.12%). Twenty-three cases (45%) showed loss of MLH1 and PMS2; 13 cases (25%) showed loss of MSH2 and MSH6; five cases (10%) showed loss of MSH6; seven cases (14%) showed loss of PMS2 and three cases (6%) showed loss of MSH2. The 2-antibody method detected 48 cases (94%) with a MMR deficiency but failed to detect three cases (6%) with an isolate loss of MSH2. The screening results from the 2-antibody method are 98.5% (200/203) in accordance with the original 4-antibody method.

Conclusion: The 2-antibody method is a quite effective option to screen for Lynch syndrome in endometrial cancers. However, MSH2 mutations may be missed in a few cases.

Keywords: Endometrial carcinoma; Lynch syndrome; MMR proteins; MSH2 loss (Siriraj Med J 2022; 74: 108-113)

INTRODUCTION

Lynch syndrome (LS) is an autosomal dominant disorder which is caused by a germline mutation in mismatch repaired (MMR) genes (MLH1, MSH2, MSH6 and PMS2) or EpCAM deletion.[1] This syndrome is associated with cancer in many organs such as the lower gastrointestinal tract, endometrium, ovary, stomach, pancreas and brain.[2] However, the two most well-known cancers associated with LS are colorectal and endometrium. Women with LS have a lifetime risk of developing colorectal cancer and endometrial cancer at 50%-85% and 40%-60%, respectively.[3,4] Although the prevalence of LS in the general population remains elusive[1], about 1.7%-5% of endometrial cancers are associated with this syndrome.[1,5-10]

For women with LS, endometrial cancer is often a sentinel tumor.[11] According to a study by Meyer et al, 61% of women with LS linked endometrial cancer had a second primary cancer, mostly colorectal cancer.[3]

Identification of LS patients is the first step in achieving proper cancer surveillance and management. Clinical

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screening criteria such as Amsterdam II and revised Bethesda guidelines have failed to detect a significant number of LS patients.[2,10,12] Thus, tumor-based testing - immunohistochemistry (IHC) for MMR proteins (MLH1, MSH2, MSH6 and PMS2) and/or microsatellite instability (MSI) - is recommended.[1-4,9,13,14]

Both IHC and MSI have a high sensitivity and specificity, however, IHC is more practical and cost effective.[3,4] In addition, MSI is less sensitive to the MSH6 germline mutation.[1,2] Many studies claim that IHC for only PMS2 and MSH6 is sufficient for initial screening[15-18] due to the binding properties of MMR heterodimer complexes; MSH2 binds with MSH6 and MLH1 binds with PMS2. With a 2-antibody approach, universal LS screening in endometrial cancers is easier to achieve, especially in places with limited resources. According to an international survey on LS screening in gynecologic cancers by Ryan et al, most pathologists still prefer the 4-antibody method.[19] In our experience and personal communication with pathologists and gynecologists, most were not confident or did not acknowledge in this cost-saving method. Moreover, most studies on a 2-antibody approach were conducted in cases of colorectal cancer. Thus, the purpose of this study was to determine the utility of the 2-antibody method in cases of endometrial cancer compared to the original 4- antibody method.

MATERIALS AND METHODS

The study was conducted at the Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand and was approved by the Siriraj Institutional Review Board (COA no. Si058/2020).

All cases of endometrial carcinoma with an immunohistochemistry conducted for the 4 MMR proteins between January 1st, 2010 and December 31st, 2019 were included in this study. Cases without available H&E and immunostained slides were excluded. Immunohistochemical staining was performed by the Ventana BenchMark ULTRA autostainer. Monoclonal antibodies for MMR proteins were as follows: anti-MLH1 (M1; Ventana), anti-PMS2 (EPR3947; Cell marque; USA), anti-MSH2 (G219-1129; Cell marque; USA) and anti-MSH6 (44; Ventana; USA). Intact expression was defined as positive nuclear staining within tumor cells. Loss of expression was defined as absence of nuclear staining within tumor cells. Stromal cells and nonneoplastic epithelial cells were used as internal control. Cases with absence of staining in internal control cells were excluded from the study. Focal and weak nuclear staining was considered as "cannot be determined".

All H&E and immunostained slides were reviewed. Results of immunohistochemistry screening were recorded and compared between all four antibodies against two (PMS2 and MSH6) antibodies. Clinical information including age at diagnosis, specimen type was retrieved from database records.

RESULTS

A total 203 cases of endometrial carcinoma with an age range of 23-62 were included in this study. Most specimens (97.54%) were from total or subtotal hysterectomy. Endometrioid carcinoma was the most common histologic subtype (89.66%). Specimen characteristics are summarized in Table 1. Loss of expression of any MMR protein was detected in 51 out of 203 cases (25.12%). Of these 51 cases with MMR deficiency, 23 cases (45%) showed loss of MLH1 and PMS2; 13 cases (25%) showed loss of MSH2 and MSH6; five cases (10%) showed loss of MSH6; seven cases (14%) showed loss of PMS2 and three cases (6%) showed loss of MSH2 (Table 2). The 2-antibody method detected 48 cases (94%) with MMR deficiency but failed to detect three cases (6%) with an isolate loss of MSH2. Isolate loss of MLH1 was not observed. One MSH2-absent/ MSH6-intact case was dedifferentiated carcinoma while the others were endometrioid type. All three cases showed convincing MSH6 expression in 20-40% of tumor cells, although the staining intensity in one case (case 2) was slightly less than internal control. (Fig 1) Overall, 98.5% (200/203) of the results from the 2-antibody method were in accordance with the original 4-antibody method.

DISCUSSION

Immunohistochemistry for MMR proteins has been acknowledged as the most practical screening test for LS and is performed routinely in many developed countries. Rates of MMR deficiency in endometrial cancer range from 19.8%- 35%.[4,7,10,12,17,18,20,21] Recently, Puangsricharoen et al reported MMR deficiency in 34.9% of 166 endometrial cancer cases in Thailand.[22] The rate of MMR deficiency in this study is 25.12% which is lower than a previous Thai study. However, population selection for this retrospective study was based on the presence or absence of immunohistochemistry for four MMR proteins and not randomized.

This study supports that IHC testing for only PMS2 and MSH6 is acceptable for initial screening. We found that PMS2 can detect all cases with loss of both MLH1 and PMS2 and PMS2 alone. While MSH6 can detect all cases with loss of both MSH2 and MSH6 and MSH6 alone. In fact, the 2-antibody method failed to identify three cases with an isolate loss of MSH2.

TABLE 1. Specimen Characteristics (n = 203).

Characteristic	Value
Age at diagnosis, average (range), years	45.06 (23-62)
Specimen type	
Total or subtotal hysterectomy	198 (97.54)
Endometrial sampling or curettage	5 (2.46)
Tumor cell type	
Endometrioid carcinoma	182 (89.66)
Endometrioid carcinoma - grade 1	96 (47.29)
Endometrioid carcinoma - grade 2	63 (31.03)
Endometrioid carcinoma - grade 3	21 (10.34)
Endometrioid carcinoma - not graded	2 (0.99)
Serous carcinoma	9 (4.43)
Mixed carcinoma	6 (2.96)
Clear cell carcinoma	3 (1.48)
Undifferentiated carcinoma	1 (0.49)
Dedifferentiated carcinoma	1 (0.49)
Carcinosarcoma	1 (0.49)

TABLE 2. Mismatch repair protein immunohistochemical staining pattern (n=203).

Immunohistochemical pattern	Number (%)
No loss of nuclear expression of MMR proteins	152 (74.88)
Loss of nuclear expression of any MMR proteins	51 (25.12)
Loss of nuclear expression of MLH1 and PMS2	23 (11.33)
Loss of nuclear expression of MSH2 and MSH6	13 (6.40)
Loss of nuclear expression of MSH6 only	5 (2.46)
Loss of nuclear expression of PMS2 only	7 (3.45)
Loss of nuclear expression of MSH2 only	3 (1.48)

Selected studies on patterns of IHC for 4 MMR proteins in endometrial cancers were reviewed (Table 3). Modica et al reported one case of isolate MSH2 loss which showed MSI-H in MSI testing.[20] Meanwhile, a study by Crim el al reported one case of isolate MLH1 loss which is impossible in the 2- antibody method, however, there was no associated germline mutation.[18] Pearlman et al also reviewed 1730 colorectal cancer cases with IHC conducted to screen for LS and reported isolate MSH2 loss in 19 cases; eight had an ambiguous MSH6 expression

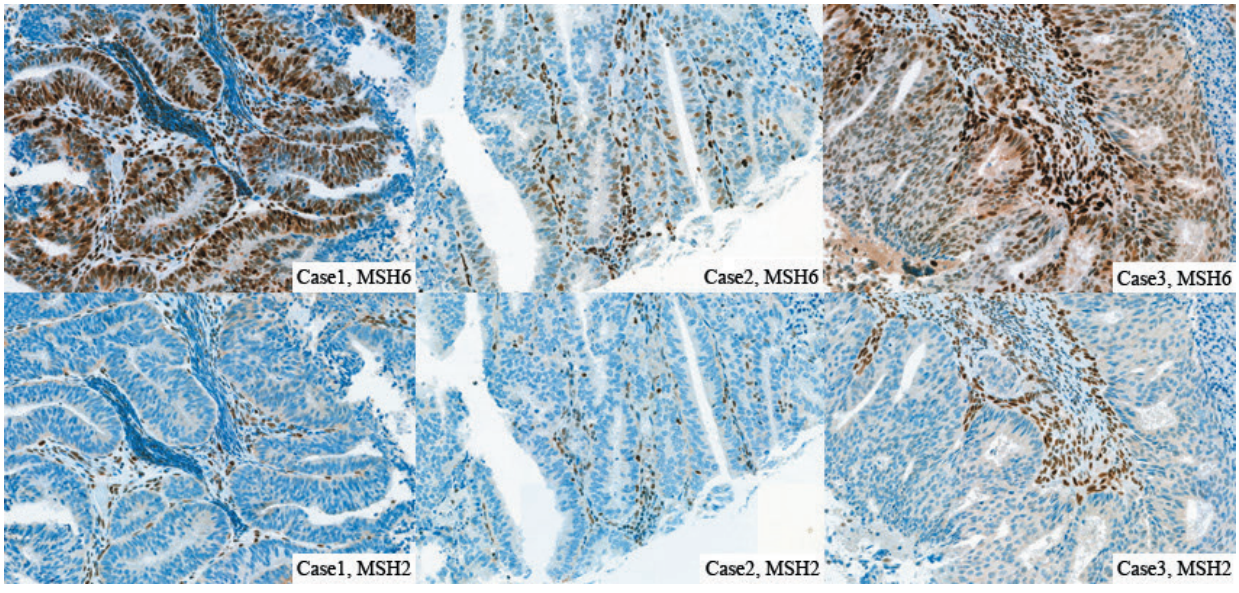

Fig 1. Three cases with an isolate loss of MSH2.

TABLE 3. Literature reports on patterns of immunohistochemical staining for MLH1, MSH2, MSH6 and PMS2 in endometrial carcinomas

Reference	Total	IHC patterns							
			Loss of any nuclear expression						
		Intact	MLH1 and PMS2	MSH2 and MSH6	MSH6 only	PMS2 only	MLH1 only	MSH2 only	Others
Modica 2007	85	37 (43.53%)	23 (27.06%)	6 (7.06%)	9 (10.59%)	6 (7.06%)	0 (0%)	1* (1.18%)	3 (3.53%)
Garg 2009	71	39 (54.93%)	19 (26.76%)	9 (12.68%)	4 (5.63%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Backes 2009	140	110 (78.57%)	24 (17.14%)	4 (2.86%)	2 (1.43%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Mojtahed 2011	40	21 (52.50%)	9 (22.50%)	4 (10%)	4 (10%)	0 (0%)	0 (0%)	0 (0%)	2 (5%)
Egoavil 2013	173	115 (66.47%)	42 (24.28%)	5 (2.89%)	7 (4.05%)	1 (0.58%)	0 (0%)	0 (0%)	3 (1.73%)
Long Q 2014	173	132 (76.30%)	10 (5.78%)	21 (12.14%)	7 (4.05%)	3 (1.73%)	0 (0%)	0 (0%)	0 (0%)
Watkins JC 2017	242	194 (80.17%)	39 (16.12%)	4 (1.65%)	3 (1.24%)	2 (0.83%)	0 (0%)	0 (0%)	0 (0%)
Crim 2017	116	92 (79.31%)	15 (12.93%)	1 (0.86%)	3 (2.59%)	2 (1.72%)	1* (0.86%)	0 (0%)	2 (1.72%)
Puangsricharoen 2020	156	99 (63.46%)	42 (26.92%)	10 (6.41%)	5 (3.21%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Our study 2021	203	152 (74.88%)	23 (11.33%)	13 (6.40%)	5 (2.46%)	7 (3.45%)	0 (0%)	3* (1.48)	0 (0%)

*cases in which the 2-antibody method could not detect defects compared to the 4-antibody method

and 11 had convincing MSH6 expression. Germline testing of these cases revealed MSH2 mutations in 7/8 cases with ambiguous MSH6 expression and 9/11 cases with convincing MSH6 expression.[23] In clinical practice, isolate MSH2 loss is unusual. Genetic consultation and further investigations, such as MSI testing or germline testing should be performed. Failure to identify these rare cases by the 2-antibody method may lead to missed opportunities for cancer surveillance and carrier testing in relatives at risk.

IHC interpretation for MMR proteins can be difficult, especially in cases with focal and weak staining. There is still no official guideline that provides the cut-off proportion and staining intensity in tumor cells. Thus, discordance results and incorrect interpretation are possible pitfalls of IHC testing.

CONCLUSION

The results from the 2-antibody method are in high accordance with the original 4-antibody method. However, the 2-antibody method fails to detect a few cases of isolate MSH2 loss which have a potential to represent those with MSH2 germline mutation.

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