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Original Article

The utility of evaluating mismatch repair proteins in endometrial carcinoma: an experience from a tertiary referral centre in North India

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Summary

Background. Endometrial cancer (EC) is a common gynecological malignancy. Around 25-30% patients have mismatch repair deficiency (MMRd). Lynch syndrome is caused by germline mutations in MMR genes. Lynch-associated tumours have better prognosis, however implications for prognosis and survival is less known. Microsatellite insufficiency (MSI) is associated with high neoantigen loads and number of tumor infiltrating lymphocytes, which overexpresses PD-1 and PD-L1 and are excellent candidates for PD-1-targeted immunotherapies. In this study, we aim to evaluate the utility of MMR in patients with EC and its clinico-pathological correlation.

Methods. Eighty-two cases of EC which underwent MMR evaluation over a period of five years at our centre were included. Demographics, clinical details including family history, histopathological and immunohistochemical (IHC) parameters were recorded. Tumors with loss-of at least one protein were considered MMR deficient (MMRd) and those with intact expression were MMR proficient (MMRp).

Results. Of 82 cases tested, 27 (33%) were MMRd. Frequencies of IHC MMR loss of expression were: MLH1/PMS2: 17 (21%), MSH6 loss only: 3 (4%), MSH2/MSH6 loss: 3 (4%), PMS2 loss: 2 (2%). In MMRd cases, most common histologic tumor type was endometrioid adenocarcinoma (70%). Loss of expression was significantly ($p < 0.001$) more frequent in lower uterine segment involvement and positive family history.

Conclusions. MSI plays an important role in the progression of endometrial cancer. Lower uterine segment involvement and positive family history are significant predictor of MMR loss. Routine testing of MMR proteins in endometrial cancer can contribute to screening of Lynch syndrome families and make immunotherapy available as a treatment option.

Key words: endometrial carcinoma, mismatch repair, lynch syndrome, microsatellite instability, immunohistochemistry

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Conflict of interest
The Authors declare no conflict of interest.

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Introduction

Endometrial cancer (EC) is one of the common gynaecological malignancies worldwide. According to ICMR, in India, the total number of estimated new cases of endometrial cancer in 2018 is 13,328 with an estimated 5010 deaths, [ICMR, 2018]. Around 20-30% of EC patients have mismatch repair deficiency [1,2]. Lynch syndrome (LS), also known as Hereditary Nonpolyposis Colorectal Carcinoma (HNPCC) is an autosomal dominant inherited cancer predisposition syndrome caused by germline mutations in MMR genes. It is associated with an increased risk of colorectal cancer (CRC), EC as well as adenocarcinomas of ovary, stomach, pancreas, and urinary tract [3]. Female relatives inheriting this mutation have a high risk of developing endometrial carcinoma, about 43% by age 75 [4].

Identification of patients with LS is important as it provides opportunity for surveillance testing, genetic counselling and risk-reducing measures to prevent LS-associated cancers [5,6]. MMR status has prognostic and predictive implications and can be used as a marker for selection of individuals for MMR genetic testing. Microsatellite instability (MSI) is associated with high neoantigen loads and an increase in the number of tumor infiltrating lymphocytes, which overexpresses PD-1 and PD-L1 and are thus excellent candidates for PD-1-targeted immunotherapies [7,8].
MMR-deficiency can be detected by either MSI analysis by polymerase chain reaction (PCR) and/or immunohistochemical staining, typically for four MMR proteins. Unlike CRC, there are no specific guidelines for screening in EC. As the lifetime risk for EC is similar to that of CRCs and that gynaecological cancers are often the sentinel malignancies diagnosed in patients with LS, MMR-IHC has been recommended to identify LS in women with ECs [9,10]. In this study, we aim to evaluate the utility of MMR in patients with EC and its clinicopathological correlation.

Materials and methods

The present study was conducted in a referral laboratory in India. Eighty-two cases of EC which underwent MMR evaluation over a period of five years were included. Demographics, clinical details, family history, histopathological and immunohistochemical parameters were recorded from the archives. In all cases, five-micron-thick sections were obtained from formalin-fixed paraffin-embedded tissue and subjected to hematoxylin and eosin (H&E) staining using standard protocols [11]. The H&E slides were evaluated through microscopic examination for tumor adequacy and processing quality. This was followed by application of a panel of immunohistochemical markers evaluation which included MLH1, MSH2, MSH6 and PMS2 using Path-n-situ monoclonal mouse antibodies against: MLH1 (clone GM011), MSH2 (clone RED2), MSH6 (EP49) and PMS2 (EP51).
Sections were deparaffinized and antigen retrieval done in pressure cooker using Tris-EDTA buffer (pH:9.0) heated to approximately 65°C. After one whistle, the cooker was switched off allowing cooling at room temperature. Buffer was changed when the temperature of buffer came to 45°C and slide put in distilled water. Endogenous peroxide was blocked using 0.3% Hydrogen peroxide in water for 15 min. Three changes for 5 min duration each washing with immunowash buffer was done (pH 7.6). The slides were incubated with primary antibody at room temperature (15-minute incubations using EnVision FLEX+ Dako Linker at room temperature). After washing, it was subjected to secondary antibodies and peroxidase (Dako EnVision for 30 minutes at room temperature). The antigen antibody reactions were visualised using DAB and counterstaining was performed using Hematoxylin. A positive control was run per batch of marker. MMR protein expression was considered positive, if ≥ 1% positive nuclei with any intensity were present; negative, if internal controls (stromal cells, benign endometrial glands and lymphocytic infiltrate) were positive and tumour cells were completely negative or showed any staining < 1% and noninformative, if tumour cells were negative and internal controls were negative. The latter may have corresponded to assay failure. Tumors with loss of at least one protein were considered MMR deficient (MMRd) and those with intact expression were MMR proficient (MMRp).
As anonymized patient data was analysed without any active intervention, the Ethical Committee approval was not needed, ethical permission was not required for the study.

Results

Of the 82 cases tested, 27 (33%) cases showed loss of expression of at least one MMR protein on IHC (MMRd) while 55 (67%) cases were found to be MMRp. The most common MMR defect identified was combined MLH1/PMS2: 17 (21%) cases. This was followed by isolated MSH6 loss: 3 (4%) cases, combined MSH2/MSH6 loss: 3 (4%) cases, isolated PMS2 loss: 2 (2%) and isolated MSH2 loss: 2 (2%) cases as shown in Figures 1 to 10. Patient characteristics according to MMR status is shown in Table I. Eight cases which were MMRd and had non-endometrioid histology included high grade serous carcinoma (n = 3), Clear cell carcinoma (n = 2), mucinous carcinoma (n = 2) and Poorly differentiated carcinoma with neuroendocrine differentiation (n = 1). It was found that there was statistically significant difference of lower uterine segment involvement and family history. On the other hand, there were no significant differences in age, histology, myometrial invasion, lymphovascular invasion, International Federation of Gynaecology and Obstetrics (FIGO) Grade, Stage and regional lymph node involvement.

Discussion

Endometrial cancer is a common malignancy worldwide with an increased incidence rate in India over

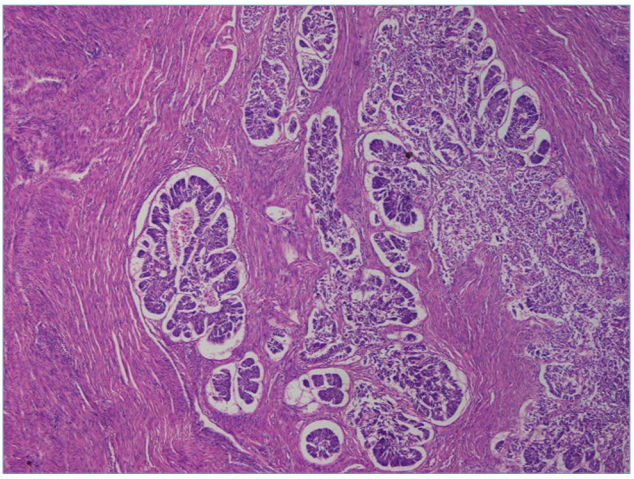

Figure 1. H and E stain showing moderately pleomorphic tumor cells arranged in complex glandular pattern in a case of Endometrioid adenocarcinoma, Grade II (x40).

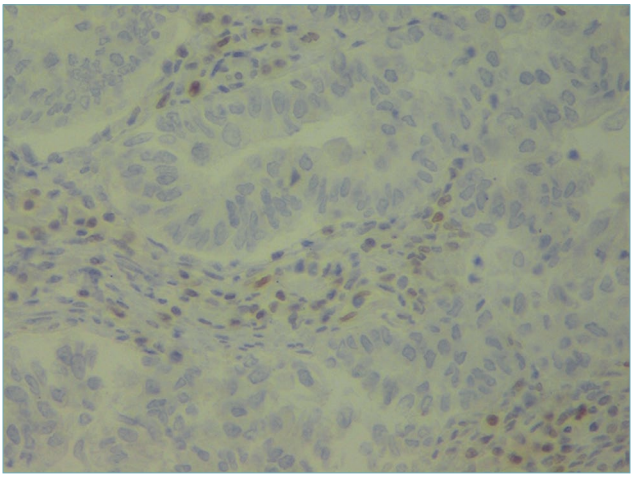

Figure 2. Loss of MLH1 expression in the case of endometrioid adenocarcinoma, Grade II (x100).

the past decade. MMR deficiency can be seen in around 20-30% of EC patients [1,2]. LS accounts for 2 to 6% of EC and women with LS have 40 to 60% lifetime risk of EC [12]. Thus, evaluation of MMR proteins in ECs help in diagnosing LS cases, detecting other LS-associated cancers, allow cascade testing of family members to identify carriers, further allowing appropriate surveillance and screening and possibly surgery to prevent cancer. MMR-IHC is a widely used method to detect MMR-deficient cases as a preliminary step as it is simple, less costly and requires less time.

Table I. Patient characteristics according to MMR protein expression.

Characteristic	MMRd cases (n = 27)	MMRp cases (n = 55)	p-value
Age (Year)	Mean: 57.9	Mean: 60.5	0.89
≤ 50 Years	06	10	
> 50 Years	21	45	
Histological type			0.82
Endometrioid	19	40	
Non-Endometrioid	08	15	
Myometrial invasion			0.23
< 50%	05	17	
> 50%	22	38	
Lymphovascular invasion			0.63
Present	12	29	
Absent	15	26	
Lower Uterine segment			0.02
Involved	21	28	
Not involved	06	27	
FIGO Grade			0.51
I/II	18	31	
III	09	24	
Stage			0.70
1/2	20	37	
3/4	07	18	
Family history			0.04
Yes	08	04	
No	19	51	
Regional lymph nodes			0.88
Involved	06	13	
Not involved	21	42	

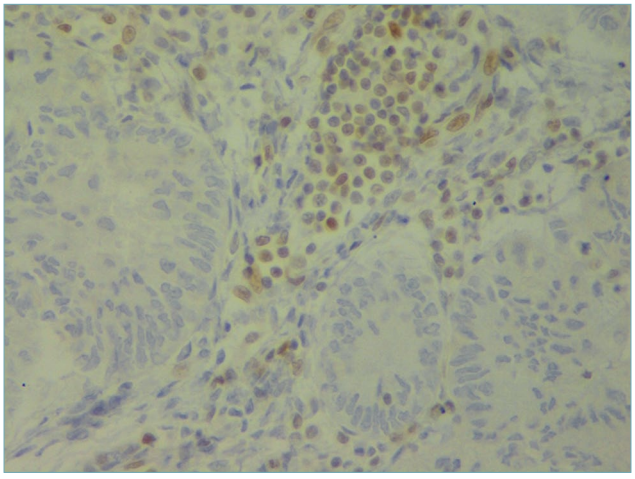

Figure 3. Loss of PMS2 expression in the case of endometrioid adenocarcinoma, Grade II (x100).

In our study, approximately 33% of EC patients showed abnormal MMR protein expression which

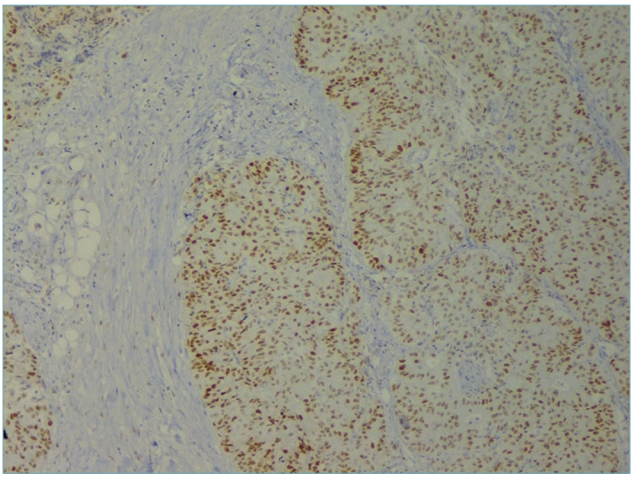

Figure 4. Intact expression of MSH2 expression in endometrioid adenocarcinoma, Grade II (x100). Stromal cells and lymphoid cells serve as internal controls.

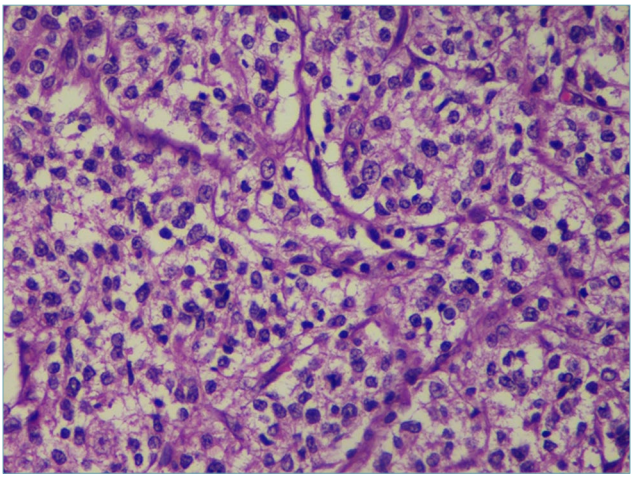

Figure 6. H and E stain showing sheets of atypical cells having hyperchromatic nuclei and clear cytoplasm in clear cell adenocarcinoma (x100).

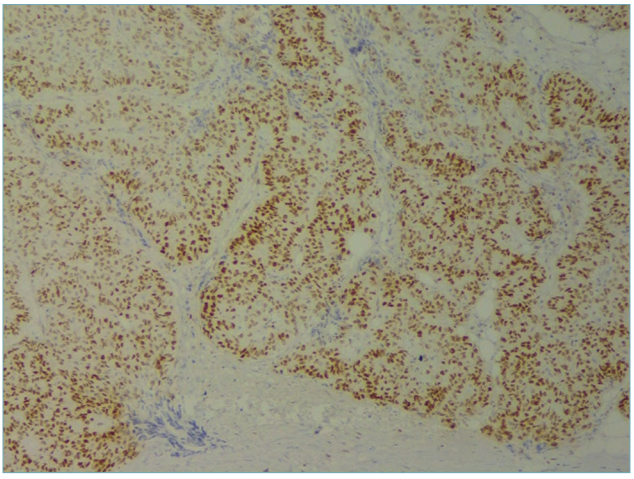

Figure 5. Intact expression of MSH6 expression in endometrioid adenocarcinoma, Grade II (x100).

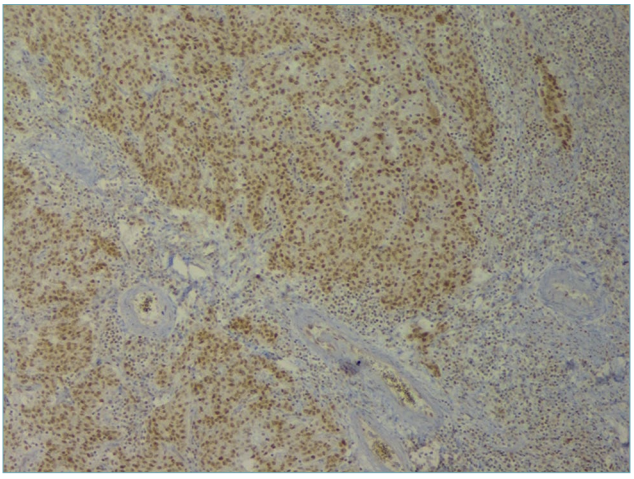

Figure 7. Intact expression of MLH1 expression in clear cell adenocarcinoma (x100).

is similar to 29% MMRd cases as reported by Buchanan DD [12]. This suggests that there is no ethnic difference of frequency in MMR-related endometrial cancers. We found slight predominance of combined loss of MLH1/PMS2 (21%) compared to another study where MSH2/MSH6 abnormalities (55.5%) were more common [13]. In current study, 19 of 27 MMRd cases (70.4%) had endometrioid histology. Previous reports have documented that approximately 67% to 94% of MMR-deficient endometrial cancers had endometrioid histology [13-15].

Around 77.8% of MMRd EC patients were found in women above 50 years of age. MMRd tumors were associated with deeper myometrial invasion, lower uterine segment involvement, low FIGO grade and presented at an early stage. These findings were similar to previously reported studies [15,16]. However, few studies have documented younger age, higher stage disease and high grade in MMRd cases [13,14,17,18].

In the present study, 5 MMRd cases had metachronous cancer (colon/rectum/ovary/breast) and 2 had recurrence. Out of these 7 cases, 4 had a positive family history. Eight patients with a family history of cancer were found to have a loss of MMR protein expression. However, 4 families with a history of cancer did not have abnormal MMR protein expression. Nine-

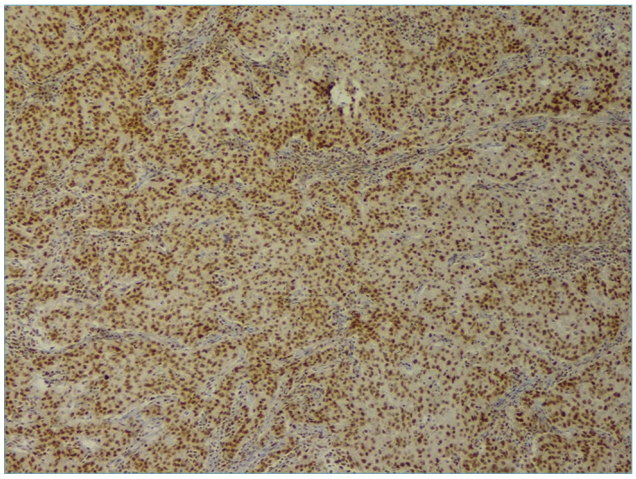

Figure 8. Intact expression of MSH2 expression in clear cell adenocarcinoma (x100).

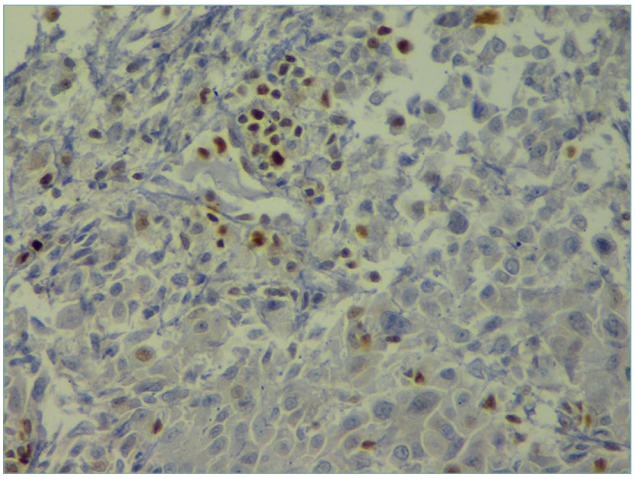

Figure 10. Loss of MSH6 expression in clear cell adenocarcinoma (x100).

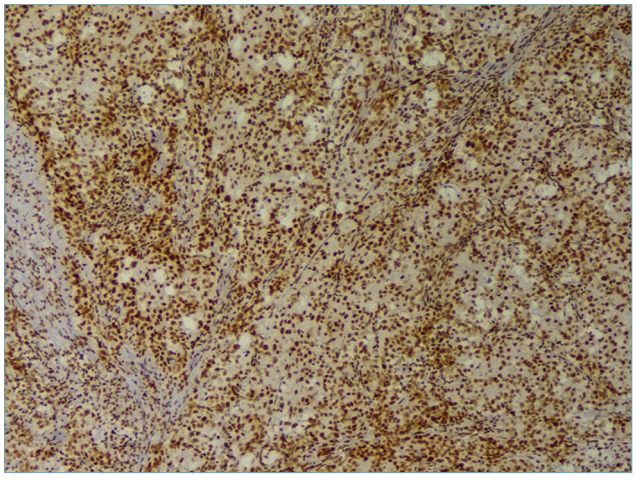

Figure 9. Intact expression of PMS2 expression in clear cell adenocarcinoma (x100).

teen patients with no family history were observed to have a loss of MMR protein expression. However, the details of follow-up for these families is not available. Buchanan DD et al proposed an algorithm for identification of germline MMR mutation carriers at diagnosis of ECs based on their observations [12]. According to them, MMR by IHC is the initial step on diagnosis of EC in patients ≤ 60 years followed by hypermethylation studies/germline mutation testing in MMRd cases for identification of germline MMR mutation carriers at diagnosis of endometrial cancers. Thus, although young patient age and family and personal histories of LS-associated cancers are reasonable criteria to select patients for screening of LS, they are insufficient, as many ECs occur in patients 50 years of age and older (as seen in the present study), it becomes important to stratify this population into smaller risk groups to restrict IHC testing to a meaningful subset of patients [19,20].

Conclusion

Microsatellite instability plays an important role in the progress of endometrial cancer. Recent studies have suggested that all EC patients should be tested for loss of MMR protein expression, regardless of age and family history. Immunohistochemical detection of MMR protein expression is a simple, economic and quick process and can guide MMR genetic mutation testing. Therefore, immunohistochemical detection of MMR protein plays an important role in screening for LS. Routine testing of MMR proteins in endometrial cancer can contribute to screening of LS families and make immunotherapy available as a treatment option.

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